Research Article

CLINICAL INVESTIGATION

Low-dose enteric-coated aspirin does not inhibit thromboxane B2 and prostaglandin E2: data-derived hypothesis formulation

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Background: All usual daily doses of plain aspirin inhibit thromboxane B2 (TXB_2) as well as prostaglandin E2 (PGE_2). The role of 81-mg enteric-coated aspirin (ECA) is controversial. **Method:** In a randomized, double-blind trial, 37 patients (25 men and 12 women) with chronic stable coronary disease taking ECA 81 mg at baseline were assigned to plain aspirin 81, 162.5, 325, 650 or 1300 mg daily for 12 weeks. At baseline and 12 weeks, blood was tested for TXB_2 and PGE_2. **Results:** All doses of plain aspirin produced virtually identical reductions in TXB_2 and PGE_2. For all doses combined, the mean ratio of the 12-week to baseline value was 0.03 for TXB_2 ($p < 0.001$) and 0.63 for PGE_2 ($p < 0.001$). **Conclusion:** These data indicate that ECA 81 mg daily does not inhibit TXB_2 and PGE_2, markers of acute and systemic responses to aspirin. Randomized trials designed *a priori* to test this hypothesis are necessary.

Keywords: enteric-coated aspirin • lack of platelet inhibition • low dose

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In secondary prevention among a wide range of patients who have survived a prior occlusive vascular event, including myocardial infarction (MI), stroke, transient ischemic attack, chronic stable angina or peripheral vascular disease, aspirin produces statistically significant and clinically important reductions in MI, stroke and cardiovascular (CV) death [1]. Specifically, in the Antithrombotic Trialist's Collaboration amongst the 170,000 randomized patients, there were no significant differences in clinical benefits in doses ranging from approximately 50 to over 1300 mg daily, the vast majority of which were plain aspirin. During acute MI [2,3] or occlusive stroke [4], plain aspirin is preferable to enteric coated aspirin (ECA), which must be crushed or chewed to achieve a rapid clinical antithrombotic effect. When given during acute MI, aspirin also produces statistically significant and clinically important benefits to MI, stroke, and CV death. In primary prevention, plain aspirin reduces the risk of a first MI, but the data on stroke and CV death remain inconclusive [5]. The most plausible mechanism for these benefits is that aspirin irreversibly inhibits platelet-dependent COX, which is responsible for the production of thromboxane B2 (TXA_2), a powerful promoter of aggregation [6]. Specifically, aspirin acetylates COX-1 causing it to be irreversibly inactivated. Since platelets lack the ability to synthesize significant amounts of protein, inactivation of COX-1 by aspirin blocks TXA_2 synthesis for the lifetime of the platelet. Platelets that do not synthesize TXA_2 normally have impaired stimulation by adenosine diphosphate, epinephrine, arachidonic acid and low doses of collagen and thrombin, but normal responses to the major platelet agonists, collagen and thrombin. Prostaglandin E2 (PGE_2) is produced by activated platelets and by several other cells, including capillary endothelial cells. PGE_2 exerts a dual effect on platelet aggregation: inhibitory at high concentrations and potentiating at

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low concentrations.

These mechanisms seem sufficient to explain the risks as well as the benefits of aspirin on clinical cardiovascular disease events. With respect to risks, gastrointestinal side effects and bleeding are well known and have been well described [1–6]. Although a dose response for gastrointestinal side effects seems apparent only at ≥325 mg daily, there has been an increasing tendency to prescribe 81 mg daily. There is also an increasing tendency to prescribe 81 mg ECA as increasing attempts to decrease gastrointestinal side effects and bleeding. Low-dose ECA has been increasingly used despite some suggestions that this dose and formulation may not produce adequate, or in some instances any, platelet inhibition [7–10]. A randomized trial was designed and conducted to test the effects of various doses of plain aspirin from 81 to 1300 mg daily, on NO as well as platelet and inflammatory biomarkers, specifically TXB_2 and PGE_2, at 12 weeks. The population studied were patients with chronic stable coronary disease, all of whom had been taking 81 mg ECA for at least one year previously. These circumstances afforded us a unique opportunity to contribute relevant data to the formulation of – but not to test – the hypothesis that low-dose ECA does not inhibit two markers of acute and systemic response to aspirin.

Method

The protocol was approved by the Institutional Review Board at Florida Atlantic University (FL, USA). All patients were recruited from two private cardiology practices. After explaining the protocol and obtaining written, informed consent for participation in a randomized, double-blind trial, 37 secondary prevention patients (25 men, 12 women) aged 46–80 years inclusive, with chronic stable coronary disease who had been prescribed daily doses of ECA 81 mg for at least 1 year previously, were enrolled in the trial. At the baseline visit, each patient was assigned at random to plain aspirin in daily doses of 81, 162.5, 325, 650 or 1300 mg for 12 weeks. All aspirin was supplied by Bayer. Compliance with aspirin during the randomized treatment and follow-up was measured by self reporting by the patients to their cardiologists and virtually all reported taking their pills at least two-thirds of the time. At baseline and 12 weeks, antecubital venous blood samples were obtained for TXB_2, the stable degradation product of TXA_2 as well as PGE_2 and a primary product of arachidonic acid metabolism. These two markers were measured on platelet-poor plasma in blood collected from antecubital veins in tubes containing EDTA. TXB_2 and PGE_2 (Cayman Chemical, MI, USA) were measured by ELISA. Each sample was measured in duplicate, and the overall intra-assay coefficients of variation were between 2.8 ± 0.3% for TXB_2 and 7.9 ± 1.2% for PGE_2, with plasma recovery rates between 87.6 and 98.9%, respectively.

Analysis of variance was used to test for the significance of differences between doses at baseline and 12 weeks, and for the paired differences between the two time points for TXB_2 and PGE_2. For all randomized daily doses of aspirin from 81 to 1300 mg, the paired differences showed virtually identical levels as well as very similar reductions in the levels of TXB_2 and PGE_2 relative to baseline. Therefore, we combined the data for all doses of aspirin and tested for statistical differences between baseline levels on 81 mg ECA and randomized dose of plain aspirin after 12 weeks using paired Student's t-tests for both TXB_2 and PGE_2. We also used paired Student's t-tests to determine whether there were significant modifications of the effects of aspirin by age (above or below the median), gender (men or women), BMI (above or below the median) and race (Caucasian, African-American or other).

For each of the two markers, TXB_2 and PGE_2, we used as a measure of strength of association the mean ratios (MRs), which we calculated as the mean level for week 12 divided by the corresponding mean level at baseline. For each MR, we calculated 95% CI by computer simulation derived from the estimated distributions of each outcome. All significance tests were conducted using a two-sided α-level of 0.05.

Role of the funding source

This trial was funded as an investigator-initiated grant by Bayer to the Charles E Schmidt College of Medicine at Florida Atlantic University, with Charles H Hennekens the Sir Richard Doll Research Professor of Medicine, as Principal Investigator. The trial was registered on the ClinicalTrials database as 'Aspirin dose and atherosclerosis in patients with heart disease'. The registration number was NCT00272337.

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Results

Despite the relatively small sample size, randomization achieved a fairly balanced distribution of baseline characteristics by treatment group (Table 1). Among the notable baseline characteristics were mean age of 64 years and mean BMI of 30.6. In addition, the vast majority of these patients with chronic stable coronary disease were being treated, according to guidelines, with statins (85%), angiotensin converting enzyme inhibitors (54%) and β-adrenergic blockers (75%).

Table 1. Baseline characteristics of randomized daily dose of aspirin treatment groups.

Baseline characteristic	Randomized daily dose of aspirin treatment groups					p-value†
	81 mg (n = 8)	162 mg (n = 7)	325 mg (n = 7)	650 mg (n = 7)	1300 mg (n = 8)	
Mean age ± SD (years)	61.8 ± 9.7	67.8 ± 8.0	61.2 ± 9.0	67.2 ± 6.0	62.6 ± 10.8	0.484
Mean height ± SD (inches)	68.3 ± 3.5	68.7 ± 3.7	65.8 ± 4.3	66.4 ± 4.2	66.3 ± 2.6	0.424
Mean weight ± SD (lb)	218.9 ± 37.7	192.4 ± 30.0	185.4 ± 60.9	202.6 ± 45.3	174 ± 28.4	0.284
Mean BMI ± SD (kg/m²)	33 ± 3.8	28.6 ± 3.2	29.7 ± 7.1	33.8 ± 5.8	27.8 ± 3.9	0.098
Caucasian (n; %)	8 (100)	6 (85.7)	6 (85.7)	7 (100)	6 (75)	0.632
PCI (n; %)	4 (50)	3 (42.9)	2 (28.6)	3 (42.9)	4 (50)	0.943
CABG (n; %)	2 (25)	3 (42.9)	1 (14.3)	1 (14.3)	3 (37.5)	0.715
Statin (n; %)	8 (100)	7 (100)	5 (71.4)	6 (85.7)	6 (75)	0.409
Other lipid lowering agent (n; %)	2 (25)	3 (42.9)	3 (42.9)	1 (14.3)	1 (12.5)	0.555
ACE inhibitor (n; %)	3 (37.5)	5 (71.4)	5 (71.4)	3 (42.9)	4 (50)	0.616
Diuretic (n; %)	2 (25)	3 (42.9)	1 (14.3)	1 (14.3)	1 (12.5)	0.715
β-blocker (n; %)	7 (87.5)	3 (42.9)	7 (100)	5 (71.4)	6 (75)	0.152
Calcium blocker (%)	1 (12.5)	5 (71.4)	2 (28.6)	1 (14.3)	1 (12.5)	0.067

Reported as mean (SD) or frequency (%).
†p-value from analysis of variance test or Fisher's exact test based on data type.
CABG: Coronary artery bypass graft; PCI: Percutaneous coronary intervention.

Of the 37 randomized patients, 33 (one missing data point for each dose except 81 mg) had complete baseline and follow-up data for TXB_2 and 36 (one missing data point for 650 mg) had complete data for PGE_2. In those patients treated with low-dose ECA at baseline, all clinically relevant doses of aspirin, including 81, 162.5, 325, 650 and 1200 mg daily, produced virtually identical and statistically significant beneficial effects on TXB_2 and PGE_2 after 12 weeks. In addition, there were no statistically significant differences between any of the doses for TXB_2 or PGE_2 either at baseline or 12 weeks; we therefore combined the data across all daily doses (Table 2).

With respect to TXB_2, there was a significant decrease (-566.7 ± 55.2; $p < 0.001$) from baseline (585.6 ± 34.4) to week 12 (19.5 ± 27.7). The MR of week 12 to baseline for TXB_2 was significantly lower than unity (0.03; 95% CI: 0.02 to 0.05; $p < 0.001$) (Table 3). In this small sample, there were no significant modifications of the effects of aspirin on TXB_2 by age, gender, BMI or race.

For PGE_2, there was a significant decrease (-132.9 ± 59.0; $p < 0.001$) from baseline (355.3 ± 49.7) to week 12 (222.4 ± 57.3), and the MR of week 12 to baseline for PGE_2 was also significantly lower than unity (0.63; 95% CI: 0.57–0.69; $p < 0.001$; Table 3). In this small sample, there were no significant modifications of the effects of aspirin on PGE_2 by age, gender, BMI or race.

Conclusion

In a randomized, double-blind trial of 37 patients with chronic stable coronary disease, who had been taking ECA 81 mg at baseline for at least 1 year prior, it was found that plain aspirin, at all doses in the usual range employed in clinical practice – including 81, 162.5, 325, 650 and 1300 mg – produced significant reductions in TXB_2 and PGE_2 after 12 weeks. These data contribute to the formulation of the hypothesis that daily administration of low dose ECA does not inhibit TXB_2 or PGE_2. These findings are compatible with several previous observations [7–10]. In one study, ECA 50 mg was not very efficient at inhibiting the arachidonic acid conversions to thromboxane as well as inhibiting *in vitro* platelet function [7]. A second, small trial showed that poor response was associated with high body weight, as well as ECA 100 mg on alternate days [8]. In another trial of 71 healthy volunteers, equivalent doses of the ECA were not as effective as plain aspirin [9]. Finally, these same investigators also showed poor response was associated with noncompliance, high body weight and use of ECA [10]. In the present trial, the mean BMI was 30.6, therefore the average patients were obese and, in addition, the sample size was too small to detect any association with even higher BMI.

There are several limitations that merit mention. First, the findings were unexpected and had not been hypothesized *a priori*. In fact, the trial was designed to test various doses of plain aspirin on NO [11], as well as platelet and inflammatory biomarkers. Thus, the

Table 2. No significant differences for thromboxane B2 and prostaglandin E2 between all clinically relevant doses of aspirin at 12 weeks compared with 81 mg enteric-coated aspirin daily at baseline.

	Randomized daily dose of aspirin				
	81 mg	162 mg	325 mg	650 mg	1300 mg
Mean 12-week-to-baseline ratios of TXB_2 (ng/ml)	0.04	0.02	0.02	0.02	0.07
Mean 12-week-to-baseline ratios of PGE_2 (ng/ml)	0.62	0.69	0.57	0.69	0.59

p-values > 0.05 for all comparisons.
PGE_2: Prostaglandin E2; TXB_2: Thromboxane B2.

findings should best be considered as data-derived hypothesis formulation. Compliance was high during the period of randomized treatment and follow-up, as measured by self reports by the patients to their private cardiologists. In addition, at the baseline visit, all patients had been prescribed ECA 81 mg for at least 1 year previously as a therapy for chronic stable coronary disease by their private cardiologists who were evaluating the patients every few months. For all these reasons, poor compliance seems less likely. Nonetheless, it is possible, at least in theory, that unrecognized and, indeed, unrecognizable bias due to noncompliance at baseline could explain the observed findings. Whether or not the patients were taking branded or generic ECA is also unknown. Despite these and other possible limitations, we believe the most plausible interpretation of the data to be that daily administration of low-dose ECA does not inhibit TXB_2 or PGE_2.

The totality of evidence on aspirin indicates that in a wide range of patients who have survived a prior occlusive vascular disease event, antiplatelet therapy principally with aspirin produces a 25% reduction in serious vascular events, such as a composite of nonfatal MI, nonfatal stroke or vascular death. In addition, there are statistically significant and clinically important benefits of aspirin for each of the components of this combined end point, specifically, nonfatal MI, nonfatal stroke or vascular death. All these patients have 10-year risks of a first coronary heart disease event of 20% or more [12]. In addition, the benefits are similar regardless of the entry criterion of the patient, including prior MI, stroke, transient ischemic attack, chronic stable angina or peripheral vascular disease. The benefits are also similar regardless of age, gender or history of hypertension or diabetes. In indirect comparisons of these trials, the benefits in reducing important vascular events by approximately 25% are similar across a wide range of doses, from 75 mg to <1500 mg of aspirin daily. Data from only three randomized trials of small sample size show that doses of >75 mg daily yield only approximately a 13% reduction, a finding that does not achieve statistical significance [1].

The issue of dose and formulation of aspirin also has relevance to the primary prevention of cardiovascular disease [13]. In that regard, prior to the Women's Health Study (WHS), five randomized trials of aspirin and their meta-analysis had been published [14]. The daily doses used in these trials ranged from 75 to 500 mg. In the meta-analysis, of amongst 55,580 apparently healthy individuals (11,466 women), there were far fewer end points than in the 287 trials of secondary prevention with 212,000 patients. Nonetheless, aspirin had a statistically significant and clinically important benefit on important vascular events, but this was due solely to a benefit on first MI of approximately a third. There were no significant benefits on stroke or vascular death, but the numbers of end points were relatively small. In four of these five trials, the 10-year risk of a first coronary heart disease event were much less than 10%, and were approximately 12.4% in the Thrombosis Prevention Trial. Specifically, the 10-year risk of a coronary heart disease event was 3.6% in the Hypertension Optimal Treatment Trial, 4.3% in the primary prevention trial, 4.8% in Physician's Health Study and 8.9% in the British Doctors' Trial. The WHS was a randomized, double-blind, placebo-controlled trial of 39,876 apparently healthy female health professionals taking aspirin 100 mg every other day for an average of 10.1 years [15]. This trial was the first to demonstrate that aspirin significantly reduced the risk of a first stroke by 17%. Furthermore, in the subgroup of women older than 65 years, who comprised approximately 10% of the study population but >30% of the end points, aspirin significantly reduced first MI (41 among women assigned to aspirin vs 62 among those assigned placebo; relative risk: 0.66; 95% CI: 0.44–0.97; p = 04). In this subgroup, aspirin also significantly reduced risk of a first ischemic stroke (53 among women assigned aspirin and 75 among those assigned placebo; relative risk: 0.70; 95% CI: 0.69–1.00; p = 0.05). As one would expect, the 10-year risk of a first coronary heart disease event in the WHS was very low; approximately 2.5%.

Table 3. Significant reductions of thromboxane B2 and prostaglandin E2 levels by aspirin at 12 weeks compared with 81-mg enteric-coated aspirin daily at baseline.

Platelet biomarker	Baseline mean ± SD (ng/ml)	12-week mean ± SD (ng/ml)	Mean 12-week to baseline concentration ratio	95% CI	Significance level (2-sided p-value)†
TXB_2	585.6 ± 34.4	19.5 ± 27.7	0.03	0.02–0.05	p < 0.001
PGE_2	355.3 ± 49.7	222.4 ± 57.3	0.63	0.57–0.69	p < 0.001

†p-value from paired T-test of change in each variable from baseline to 12 weeks.
PGE_2: Prostaglandin E2; TXB_2: Thromboxane B2.

In a landmark clinical investigation of patients with unstable angina, as well as in apparently healthy volunteers, a dose of 75 mg/day of plain aspirin was sufficient to achieve complete inhibition of TXB_2 [16]. The time course of this effect, however, was approximately 2 days. Thus, in secondary prevention for patients who have survived a wide range of prior occlusive events, as well as in primary prevention, doses of 75–325 mg/day have been recommended. In acute MI, however, an initial loading dose of 162.5–325 mg is required to achieve a rapid clinical antithrombotic effect. Few trials have directly compared higher versus lower doses of aspirin. Indirect comparisons of data from different trials are useful to formulate hypotheses, but any observed findings could be due to differences in the population studied. Nonetheless, in indirect comparisons of data from various randomized trials, higher doses of 500–1500 mg/day are no more effective than medium doses of 162.5–325 mg or low doses of 75–162.5 mg. No indirect evidence supports the suggestion that aspirin doses of >1000 mg/day might be preferable for the prevention of serious vascular events among patients at high risk of stroke. Finally, although based on data from only three randomized trials before the publication of the WHS, daily doses of <75 mg seem to have a somewhat smaller and nonsignificant effect. Specifically, the proportional reduction of vascular events was 19, 26, 32 and 13% with doses of 500–1500, 162.5–325, 75–150 and <75 mg/day, respectively [1]. More recently, however, the WHS demonstrates clinical benefits of 100 mg of aspirin on alternate days [14]. Most of these trials used plain aspirin. In addition, ISIS-2 used 162.5 mg ECA, but wisely instructed all clinicians and their patients to be certain that all tablets were crushed or chewed to achieve a rapid clinical antithrombotic effect [2].

At present, lower doses of plain aspirin as well as ECA are being used in an effort to avoid gastrointestinal and bleeding side effects as well as to preserve prostacyclin formation. With respect to gastrointestinal side effects, in the UK trial of transient ischemic attacks, a randomized double-blinded, placebo-controlled trial of 5 years duration, the rates were 29% in the 325 mg daily aspirin group and 25% in the placebo group, so the rate attributable to aspirin was approximately 4% [17]. As regards significant bleeding, the corresponding rates were 2.6 and 1.6%, respectively, so the rate attributable to aspirin was approximately 1%. In addition, the clinical relevance of prostacyclin sparing has never been demonstrated, as the clinical benefits of aspirin are present across a wide range from low doses used predominantly in treatment trials of coronary patients and higher doses used predominantly in treatment trials of stroke patients.

In summary, the current data further contribute to the the hypothesis that patients who use low-dose ECA preparations may not attain the clinical benefit on occlusive CVD events. If this hypothesis were true, then it is tempting to speculate that use of higher doses of ECA or plain aspirin at a daily dose of 81 mg would resolve the issue in clinical practice. Lower bioavailability of ECA preparations and poor absorption from the higher pH environment of the small intestine may result in inadequate platelet inhibition. With respect to bioavailability, there may be important differences between the branded products and the over-the-counter generic ECA. Randomized trials of sufficient size designed *a priori* to test these hypotheses are necessary and should include various doses of generic and branded plain aspirin as well as equivalent doses of ECA.

Future perspective

These findings contribute important relevant information to the hypothesis that 81 mg ECA daily does not inhibit TXB_2 and PGE_2 markers of acute and systemic responses to aspirin. Randomized trials of sufficient size designed *a priori* to test these hypotheses are necessary and should include various doses of generic and branded plain aspirin, as well as equivalent doses of ECA. If this hypothesis is correct, the next 5–10 years may see a paradigm shift toward the utilization of higher doses of ECA in the treatment and prevention of CVD. It is also plausible that greater utilization of 325 mg daily may occur if the randomized data in prevention of colon polyps as well as colon cancer are added to the benefit–risk considerations for daily aspirin use.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal

experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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