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ORIGINAL INVESTIGATIONS

Enteric Coating and Aspirin Nonresponsiveness in Patients With Type 2 Diabetes Mellitus

Deepak L. Bhatt, MD, MPH,[a] Tilo Grosser, MD,[b] Jing-fei Dong, MD, PhD,[c] Douglas Logan, MD,[d] Walter Jeske, PhD,[e] Dominick J. Angiolillo, MD, PhD,[f] Andrew L. Frelinger III, PhD,[g] Lanyu Lei, MSc,[h] Juan Liang, PhD,[i] Jason E. Moore, MS,[j] Byron Cryer, MD,[k] Upendra Marathi, PhD[j]

ABSTRACT

BACKGROUND A limitation of aspirin is that some patients, particularly those with diabetes, may not have an optimal antiplatelet effect.

OBJECTIVES The goal of this study was to determine if oral bioavailability mediates nonresponsiveness.

METHODS The rate and extent of serum thromboxane generation and aspirin pharmacokinetics were measured in 40 patients with diabetes in a randomized, single-blind, triple-crossover study. Patients were exposed to three 325-mg aspirin formulations: plain aspirin, PL2200 (a modified-release lipid-based aspirin), and a delayed-release enteric-coated (EC) aspirin. Onset of antiplatelet activity was determined by the rate and extent of inhibition of serum thromboxane B_2 (TXB_2) generation. Aspirin nonresponsiveness was defined as a level of residual serum TXB_2 associated with elevated thrombotic risk (<99.0% inhibition or TXB_2 >3.1 ng/ml) within 72 h after 3 daily aspirin doses.

RESULTS The rate of aspirin nonresponsiveness was 15.8%, 8.1%, and 52.8% for plain aspirin, PL2200, and EC aspirin, respectively ($p < 0.001$ for both comparisons vs. EC aspirin; $p = 0.30$ for comparison between plain aspirin and PL2200). Similarly, 56% of EC aspirin–treated subjects had serum TXB_2 levels >3.1 ng/ml, compared with 18% and 11% of subjects after administration of plain aspirin and PL2200 ($p < 0.0001$). Compared with findings for plain aspirin and PL2200, this high rate of nonresponsiveness with EC aspirin was associated with lower exposure to acetylsalicylic acid (63% and 70% lower geometric mean maximum plasma concentration [C_{max}] and 77% and 82% lower AUC_{0-t} [area under the curve from time 0 to the last time measured]) and 66% and 72% lower maximum decrease of TXB_2, with marked interindividual variability.

CONCLUSIONS A high proportion of patients treated with EC aspirin failed to achieve complete inhibition of TXB_2 generation due to incomplete absorption. Reduced bioavailability may contribute to "aspirin resistance" in patients with diabetes. (Pharmacodynamic Evaluation of PL2200 Versus Enteric-Coated and Immediate Release Aspirin in Diabetic Patients; NCT01515657) (J Am Coll Cardiol 2017;69:603–12) © 2017 by the American College of Cardiology Foundation.

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From the [a]Brigham and Women's Hospital Heart and Vascular Center and Harvard Medical School, Boston, Massachusetts; [b]Institute for Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia, Pennsylvania; [c]Hematology Division, Department of Medicine, University of Washington School of Medicine, Seattle, Washington; [d]Veterans Administration Medical Center, Cincinnati, Ohio; [e]Loyola University Chicago, Cardiovascular Research Institute, Maywood, Illinois; [f]University of Florida College of Medicine, Jacksonville, Florida; [g]Center for Platelet Research Studies, Division of Hematology/Oncology, Boston Children's Hospital, Dana Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; [h]Harvard Clinical Research Institute, Boston, Massachusetts; [i]Medpace Inc., Cincinnati, Ohio; [j]PLx Pharma Inc., Houston, Texas; and the [k]University of Texas Southwestern and VA North Texas Health Care System, Dallas, Texas. This study was funded by PLx Pharma Inc. Dr. Bhatt has served on the advisory board for Cardax, Elsevier Practice Update Cardiology, Medscape Cardiology, and Regado Biosciences;

ABBREVIATIONS AND ACRONYMS

AA = arachidonic acid

ASA = acetylsalicylic acid

AUC_{0-t} = area under the curve from time 0 to the last time measured

C_{max} = maximum plasma concentration

C_{min} = minimum concentration

COX = cyclooxygenase

EC = enteric-coated

PD = pharmacodynamic

PK = pharmacokinetic

$T_{99\%inhibition}$ = time to ≥99% inhibition of serum thromboxane B_2

TX = thromboxane

TXB_2 = thromboxane B_2

For several years, aspirin "resistance" has been a controversial topic. Certainly, there are patients who are taking aspirin for cardiovascular protection who nevertheless experience a platelet-mediated ischemic event. In some proportion of those cases, it may indeed be considered a clinical failure of aspirin, whereas in other cases, alternative pathological mechanisms may have been at play (1). Some patients who do not seem to benefit from aspirin do not adhere to the recommended aspirin regimen for a variety of reasons, including gastric intolerance or bleeding (2,3). Pharmacological failure of aspirin, in which inadequate inhibition of its drug target, platelet cyclooxygenase (COX)-1, is documented by thromboxane (TX) measurement, seems to be infrequent (<6%) (4). Suppression of TX is believed to explain aspirin's cardioprotective benefit mechanistically (5).

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Both aspirin dosing and enteric coating have been suggested to influence the antiplatelet effects of aspirin. No convincing clinical trial evidence exists that the dose level or scheduling of aspirin affects clinical outcomes in the chronic cardiovascular setting, although some data suggest that patients with diabetes mellitus may display less aspirin resistance with higher doses (i.e., 325 mg vs. 81 mg) or more frequent dosing intervals (twice daily vs. once daily) (6–9). The clinical effect of enteric-coated (EC) aspirin versus uncoated ("plain") aspirin on cardiovascular events has never been fully studied. Some data suggest greater variability of antiplatelet response to EC aspirin (10–12). A study of healthy volunteers found that all detected aspirin nonresponsiveness was attributable to the enteric coating, presumably due to lower formulation-dependent bioavailability (12).

In addition, aspirin is known to cause gastrointestinal discomfort and bleeding. However, it is not clear that the enteric coating actually reduces clinically significant gastrointestinal events, such as bleeding or ulceration (13). A lipid-based formulation of aspirin (PL2200) has been shown in a randomized trial to reduce the risk of acute endoscopic ulceration compared with plain aspirin (14). We therefore conducted a study of plain aspirin versus PL2200 versus EC aspirin to determine the influence of aspirin formulation on the drug response by monitoring the rate and extent of TX suppression, platelet aggregation, and absorption of aspirin.

METHODS

STUDY DESIGN. This study was conducted at the Medpace Clinical Pharmacology Unit, Cincinnati, Ohio. The study was a single-center, randomized, active-control, single-blinded (all study staff except

board of directors for Boston VA Research Institute and Society of Cardiovascular Patient Care; chair for the American Heart Association Quality Oversight Committee; data monitoring committees for Duke Clinical Research Institute, Harvard Clinical Research Institute, Mayo Clinic, and Population Health Research Institute; has received honoraria from the American College of Cardiology (Senior Associate Editor, *Clinical Trials and News*, ACC.org), Belvoir Publications (Editor in Chief, *Harvard Heart Letter*), Duke Clinical Research Institute (clinical trial steering committees), Harvard Clinical Research Institute (clinical trial steering committee), HMP Communications (Editor in Chief, *Journal of Invasive Cardiology*), *Journal of the American College of Cardiology* (Guest Editor and Associate Editor), Population Health Research Institute (clinical trial steering committee), Slack Publications (Chief Medical Editor, *Cardiology Today's Intervention*), Society of Cardiovascular Patient Care (Secretary/Treasurer), and WebMD (CME steering committees); served as Deputy Editor for *Clinical Cardiology*; served as chair for the NCDR-ACTION Registry Steering Committee, and VA CART Research and Publications Committee; has received research funding from Amarin, Amgen, AstraZeneca, Bristol-Myers Squibb, Eisai, Ethicon, Forest Laboratories, Ischemix, Lilly, Medtronic, Pfizer, Roche, Sanofi, and The Medicines Company; royalties from Elsevier (Editor, *Cardiovascular Intervention: A Companion to Braunwald's Heart Disease*); has served as a site co-investigator for Biotronik, Boston Scientific, and St. Jude Medical; has served as a trustee for the American College of Cardiology; and has performed unfunded research for FlowCo, PLx Pharma Inc., and Takeda. Dr. Grosser has received consulting fees from PLx Pharma Inc., Bayer Healthcare, and Aralez Pharmaceuticals; and is an Associate Editor for *Circulation: Cardiovascular Genetics*. Dr. Angiolillo has received payment as an individual for consulting fee or honorarium from Sanofi, Daiichi-Sankyo, Inc., Eli Lilly and Company, The Medicines Company, AstraZeneca, Merck, Abbott Vascular, Amgen, Bayer, Pfizer, and PLx Pharma; participation in review activities from CeloNova, Johnson & Johnson, and St. Jude Medical; and institutional payments for grants from GlaxoSmithKline, Daiichi-Sankyo, Inc., Eli Lilly and Company, The Medicines Company, AstraZeneca, Janssen Pharmaceuticals, Inc., Osprey Medical, Inc., Novartis, CSL Behring, and Gilead. Dr. Jeske is the principal investigator on a research grant to Loyola University Chicago from BioData Corporation; and is a consultant to PLx Pharma Inc., Machaon Diagnostics, and Repros Therapeutics. Dr. Frelinger is the principal investigator or co-investigator on research grants to Boston Children's Hospital from Baxalta, Bristol-Myers Squibb, Eisai, Eli Lilly, Daiichi-Sankyo, GE Healthcare, GLSynthesis, Pfizer, and Sysmex; and is a consultant to PLx Pharma. Mr. Moore is an officer and employee of PLx Pharma Inc. Dr. Marathi is an investor, officer, and employee of PLx Pharma Inc.; is the co-inventor of the PL2200 technology; and holds equity and options to buy equity in 7 Hills Pharma. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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patients), triple-crossover, pharmacokinetic (PK), and pharmacodynamic (PD) trial. It compared the onset and offset of antiplatelet activity after treatment with immediate-release aspirin tablets (Genuine Bayer Aspirin; Bayer, Whippany, New Jersey), PL2200 aspirin capsules (PLx Pharma Inc., Houston, Texas), and EC aspirin caplets (Safety-Coated Aspirin; Bayer) at a dose of 325 mg once daily for 3 days in obese patients with type 2 diabetes mellitus with no history of cardiovascular disease (Online Figure 1). Each dose of study drug was administered with 8 ounces of water by the blinded clinic staff, after an overnight 10-h fast, and the subjects were fed a standardized meal 2 h after administration of each dose. After the 2-day treatment phase, laboratory assessments continued on days 4 to 7. After a 2-week washout period, the next drug was tested and the process repeated again in each patient.

To ensure study compliance, patients were admitted to the Phase I clinic for 2 nights for all baseline and initial dose PK and PD assessments. All subsequent dosing and sampling were conducted on an outpatient basis. The compliance to study drugs for the 3 periods was 100%.

STUDY POPULATION. This study evaluated the aspirin PK and PD parameters in obese patients with diabetes because weight and poor glycemic control have been suggested as important risk factors for suboptimal aspirin PK and PD findings (15,16). Obese subjects age 21 to 79 years, with a body mass index of 30 to 40 kg/m^2, with no history of vascular disease and who had type 2 diabetes mellitus not requiring insulin were eligible for the study. A glycosylated hemoglobin level >6.4% and/or fasting plasma glucose level >125 mg/dl at screening or current antidiabetic medication use confirmed the diagnosis of diabetes. Nonsteroidal anti-inflammatory drugs, antisecretory agents, antacids, and salicylate-containing nutritional supplements were not allowed within 2 weeks of randomization. To ensure normal platelet function, all patients had an arachidonic acid (AA)-induced platelet aggregation response of >60% aggregation within 3 h before administration of the initial dose of study drug for each period.

Serum TX. The onset and offset of inhibition of platelet COX-1 by aspirin was evaluated by measuring inhibition of ex vivo-generated serum thromboxane B_2 (TXB_2) (17–19). Briefly, immediately after approximately 4 ml of blood was drawn, the non-anticoagulated blood specimen was incubated for 1 h at 37°C, the resulting serum was isolated, and it was stored frozen until analysis. TXB_2 was quantified by using a validated high-performance liquid chromatography-tandem mass spectrometry assay (MEDTOX Scientific, St. Paul, Minnesota). The lower limit of quantitation of the method was 1.00 ng/ml using d_4-TXB_2 as the internal standard.

TABLE 1 Baseline Demographic Characteristics (N = 40)

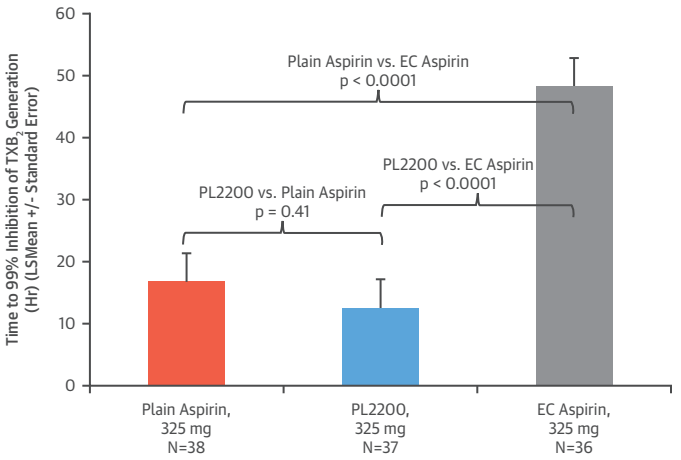
Age, yrs	52.95 ± 10.12
Male	65.0
Glycosylated hemoglobin, %	7.24 ± 1.03
Fasting glucose, mg/dl	132.78 ± 27.14
Weight, kg	101.53 ± 14.38
Body mass index, kg/m^2	34.45 ± 2.72

Values are mean ± SD or %.

Pharmacokinetics. Acetylsalicylic acid (ASA) and salicylic acid were analyzed by using a validated high-performance liquid chromatography-tandem mass spectrometry method for salicylates (MEDTOX Scientific). The validated method has a lower limit of quantitation of 50.0 ng/ml, using d_4-salicylic acid and d_4-ASA as the respective internal standards. Both salicylate and TXB_2 analytical methods were fully validated according to current industry standards for bioanalytical testing, as defined by the US Food and Drug Administration for accuracy and precision.

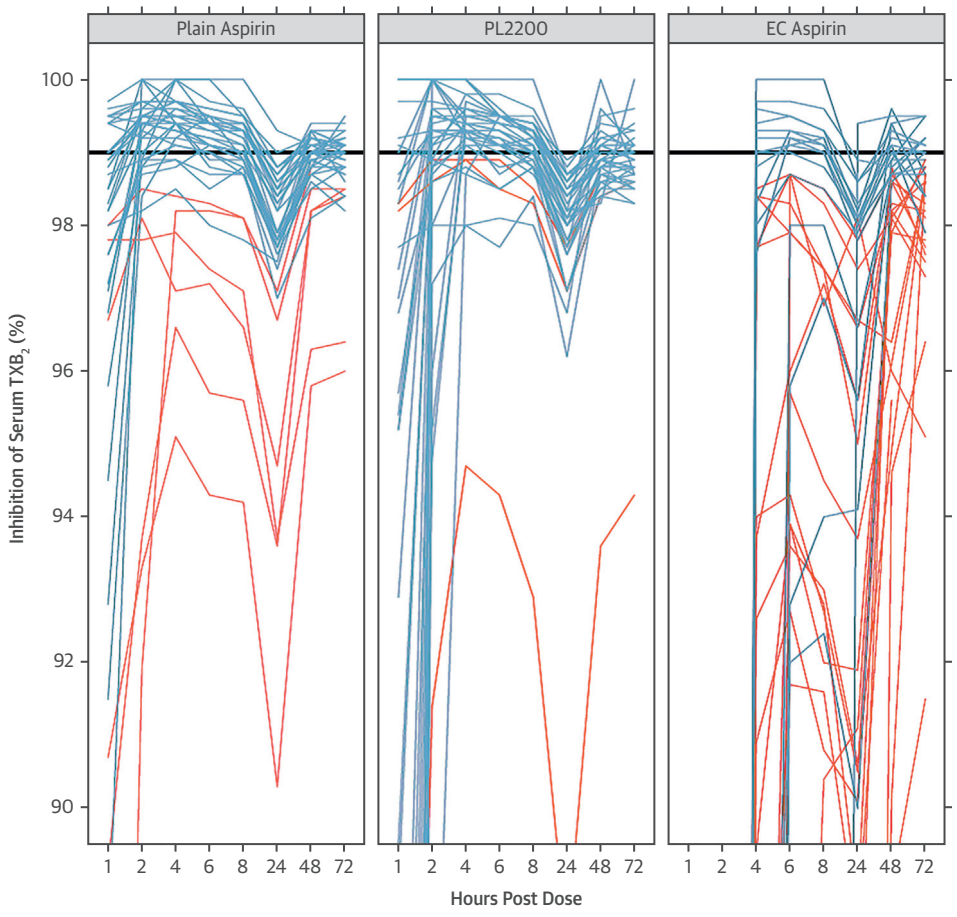
Platelet aggregation. Platelet aggregation was determined in platelet-rich plasma by turbidimetry. Processing of the platelet-rich plasma was initiated within 15 min of blood draw. Platelet aggregation was induced at 37°C with constant stirring (1,000 rpm) by AA (0.5 mg/ml; Bio/Data Corp., Horsham,

FIGURE 1 PL2200 and Plain Aspirin Have Faster Onsets of Complete Aspirin Response



The p values were assessed by using the mixed effects model. A faster onset of response was seen with plain aspirin or PL2200 (a modified-release lipid-based aspirin) compared with enteric-coated (EC) aspirin, as assessed by time to 99% inhibition of thromboxane B_2 (TXB_2) generation. LS = least-squares.

FIGURE 2 EC Aspirin Has a Lower Extent and Highly Variable Depletion of TXB_2



Percent inhibition of serum TXB_2 concentration over baseline after dosing with plain aspirin, PL2200, and EC aspirin. **Blue lines** represent % inhibition in individual patients who reached 99% inhibition by 72 h after the first dose of study drug. **Orange lines** represent % inhibition in individual patients who did not reach 99% inhibition by 72 h after the first dose of study drug. The **horizontal line** designates 99% inhibition. Abbreviations as in Figure 1.

Pennsylvania) or type I fibrillar collagen (4 μg/ml; Helena Laboratories, Beaumont, Texas) on an optical platelet aggregometer (Model 700; Chrono-Log Corp., Havertown, Pennsylvania). At screening and before crossover, the samples that exhibited <60% AA aggregation were retested. The platelet counts for the collagen aggregation assay were standardized to 2 to 2.5×10^5/μl using autologous platelet-poor plasma.

Aspirin response criteria. A complete aspirin response was determined on the basis of 2 thresholds for TXB_2 depletion that seem to stratify long-term cardiovascular risk: ≥99.0% inhibition of TXB_2 formation over baseline (20,21) or a nonbaseline-adjusted criterion of 3.1 ng/ml, on the basis of Frelinger et al. (22). Nonresponsiveness was defined as patients who did not reach 99% inhibition of TXB_2 formation or a minimum concentration (C_{min}) of >3.1 ng/ml at any time during the first 72 h (3 doses) of the study.

STATISTICAL ANALYSIS. The Harvard Clinical Research Institute (Boston, Massachusetts) performed an independent statistical analysis. Data were summarized by using descriptive statistics (sample size [n], mean, median, SD, minimum and maximum values for continuous variables, and frequency and percentage of patients for categorical variables). All mixed effect models included sequence, period, and treatment as fixed effects, and patient as a random effect.

PD ANALYSES. Onset endpoints. The primary endpoint for this study was time to ≥99% inhibition of serum TXB_2 ($T_{99\%inhibition}$), testing for superiority of PL2200 over EC aspirin through 72 h after the first dose. The study was designed to detect a significant difference

between $T_{99\%inhibition}$ for EC aspirin and PL2200 with 90% power. Estimates of $T_{99\%inhibition}$ for PL2200 and plain aspirin were on the basis of a prior pilot crossover PK and platelet function study in healthy volunteers, and estimates for EC aspirin are from Grosser et al. (12). $T_{99\%inhibition}$ was analyzed by using a mixed effects model, with sequence, period, and treatment as fixed effects, and patient as a random effect.

Secondary endpoints included incidence of aspirin responders on the basis of $T_{99\%inhibition}$ or >3.1 ng/ml, on percent inhibition of maximum AA-induced platelet aggregation, or on percent inhibition of maximum collagen-induced platelet aggregation. The bioavailability of ASA with respect to maximum plasma concentration (C_{max}) and area under the curve from time 0 to the last time measured (AUC_{0-t}) was analyzed by using a mixed effects models with sequence, period, and treatment as fixed effects, and patient as a random effect. The marginal homogeneity of TXB_2 nonresponsiveness was evaluated by using McNemar's test.

Offset endpoints. Offset was evaluated categorically by measuring time to ≤95% inhibition of serum TXB_2 over baseline, and calculating the rate of TXB_2 recovery after the last dose.

RESULTS

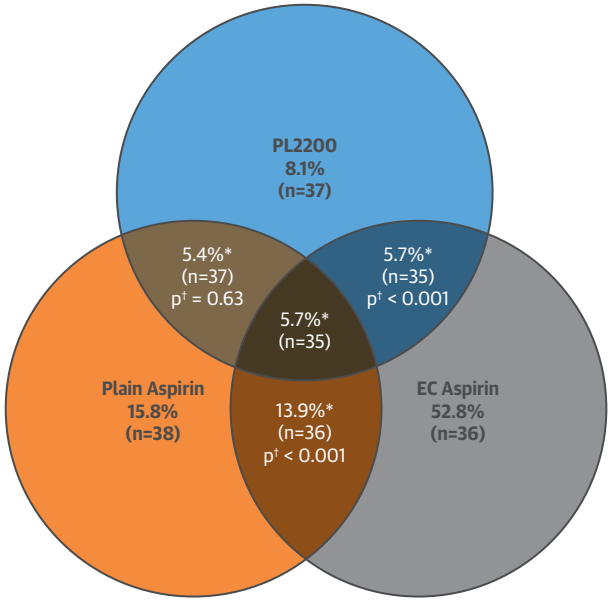
A total of 40 patients were enrolled in the study; 35 (87.5%) completed all 3 crossover phases. The reasons for dropout were not related to the study drug (2 cases of acute gout, 1 tooth abscess, 1 prohibited medication, and 1 withdrawal of consent). Their baseline characteristics are listed in Table 1. The median age was 54 years, and 65% were men. The median glycosylated hemoglobin level was 7%, and the median body mass index was 34.35 kg/m^2.

ONSET OF ASPIRIN ANTIPLATELET ACTIVITY. Assessment of aspirin nonresponsiveness by depletion of serum TX. The rate and extent of inhibition of platelet COX-1 activity over 3 daily doses of 325 mg aspirin and the subsequent recovery of such activity were assessed by measuring platelet capacity to generate TXB_2. Aspirin responsiveness was evaluated by using 2 criteria that may predict long-term cardiovascular risk: time-to-event and incidence of patients reaching 99% inhibition or <3.1 ng/ml over 3 daily doses.

The primary endpoint for the study, to assess superiority of PL2200 relative to EC aspirin for the time required to achieve $T_{99\%inhibition}$, was met. The times to a complete aspirin response for plain aspirin, PL2200, and EC aspirin were 16.7 ± 4.5 h (n = 38), 12.5 ± 4.6 h (n = 37), and 48.2 ± 4.6 h (n = 36), respectively (Figure 1). Plain aspirin resulted in significantly faster TXB_2 inhibition than EC aspirin ($p < 0.0001$). PL2200 achieved 99% TXB_2 inhibition significantly faster than EC aspirin ($p < 0.0001$), and the PL2200 rate was similar to that of plain aspirin ($p = 0.41$).

Individual patient responsiveness during the onset period is illustrated in Figure 2. PL2200 or plain aspirin resulted in a greater extent of inhibition of TXB_2 generation that was less variable than that observed after administration of EC aspirin. The incidence of incomplete response was significantly lower after administration of PL2200 than after EC aspirin ($p < 0.0001$) (Central Illustration) but similar to that after plain aspirin. In confirming these findings, the incidence of patients with the lowest observed TXB_2 (C_{min}) >3.1 ng/ml TXB_2 for plain aspirin (n = 38) or PL2200 (n = 37) was 18.4% and 10.8%, respectively; these findings were both significantly lower than when the subjects were crossed over to EC aspirin

CENTRAL ILLUSTRATION Intraindividual Risk of Nonresponsiveness Is Formulation-Dependent



Bhatt, D.L. et al. J Am Coll Cardiol. 2017;69(6):603–12.

The figure illustrates the incidence of aspirin nonresponsiveness after dosing with plain aspirin, PL2200 (a modified-release lipid-based aspirin), and enteric-coated (EC) aspirin. The nonoverlapping portion of each circle indicates the incidence of nonresponsiveness after treatment with each of the 3 aspirin formulations. The overlapping portions indicate the proportion of patients that are nonresponsive to 2 or more formulations. *The 2 subjects nonresponsive to all drugs are the same 2 subjects in 5.7% between PL2200 and EC Aspirin, and the same 2 subjects in 5.4% between PL2200 and Plain Aspirin, and part of the 5 subjects in 13.9% between Plain Aspirin and EC Aspirin. †The p values were assessed by using McNemar's test.

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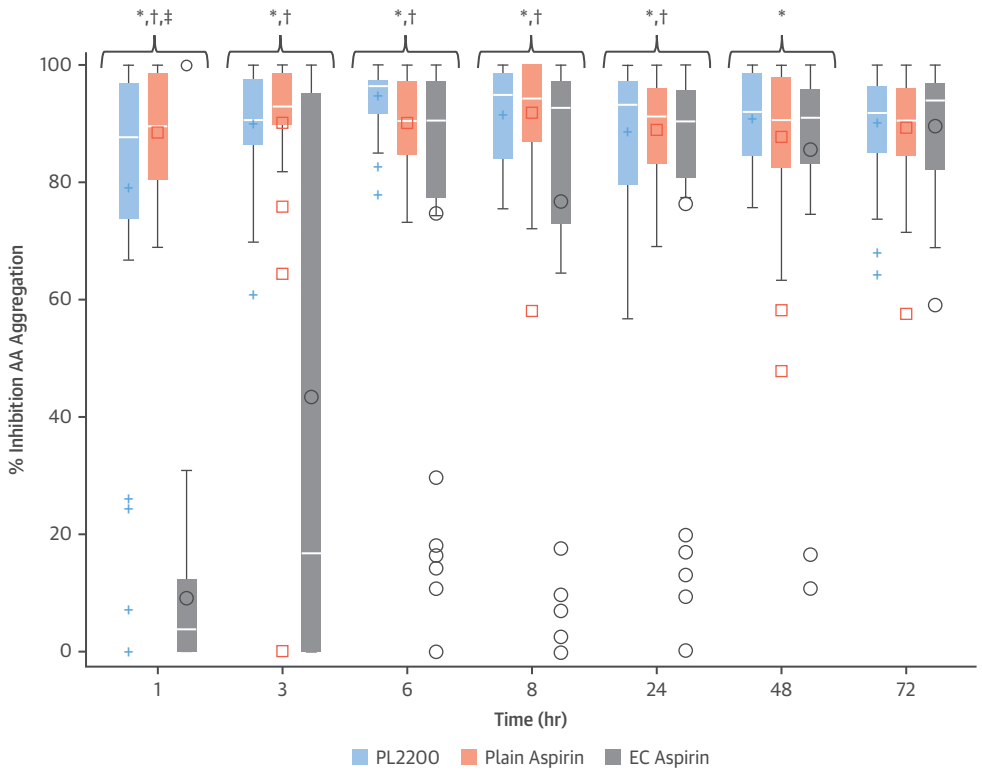
FIGURE 3 Differences Among the 3 Aspirin Formulations in Inhibition of AA-Induced Platelet Aggregation



Inhibition of arachidonic acid (AA)-induced platelet aggregation shown by light transmittance aggregometry after administration of PL2200 (n = 38), plain aspirin (n = 38), and EC aspirin (n = 37). Least-squares mean ± SE are presented: *EC aspirin versus PL2200, $p < 0.05$; †EC aspirin versus plain aspirin, $p < 0.05$; ‡PL2200 versus plain aspirin, $p < 0.05$. The p values were assessed by using the mixed effects model. The differences and distributions for each treatment at each time point are displayed by box plots. **Boxes** are defined by the lower quartile and the upper quartile, and the **line** goes through the median; the **plus, square, and circle symbols inside the boxes** are the mean values. **Whiskers below and above the box** are defined by 1.5 times the interquartile range. Observations outside the **whiskers** are considered as outliers. Abbreviations as in Figure 1.

(55.6%, n = 36; $p < 0.001$). Interestingly, C_{min} values observed after a single dose of plain aspirin and PL2200 (2.18 ± 1.74 ng/ml and 1.81 ± 1.53 ng/ml) were significantly lower than the C_{min} for EC aspirin (6.35 ± 8.13 ng/ml), even after 3 doses.

Both responsiveness criteria seem consistent, as 16 of 20 EC aspirin-treated patients with >3.1 ng/ml TXB_2 had <99% inhibition of TXB_2. Only 2 subjects (5.7%) failed to respond to any formulation of aspirin according to the study definition of ≥99.0% inhibition of TXB_2

TABLE 2 Comparison of Acetylsalicylic Acid Pharmacokinetics

	Plain Aspirin 325 mg (n = 35)	PL2200 325 mg (n = 37)	EC Aspirin 325 mg (n = 36)	p Value* of PL2200 vs. EC Aspirin	p Value* of Plain Aspirin vs. EC Aspirin	p Value* of PL2200 vs. Plain Aspirin
C_{max}, ng/ml Geometric LS	1,442.47 (35)	1,803.18 (37)	538.93 (29)	<0.0001	<0.0001	0.2538
AUC_{0-t}, ng × h/ml Geometric LS	1,963.7 (35)	2,523.1 (37)	455.8 (29)	<0.0001	<0.0001	0.1375
T_{max}, h	1.1 ± 0.4 (35)	1.3 ± 0.6 (37)	3.5 ± 1.2 (29)	<0.0001	<0.0001	0.3275

Values are mean (N) or mean ± SD (N). *The p values were assessed by using a mixed effects model, with sequence, period, and treatment as fixed effects, and patient as a random effect.

AUC_{0-t} = area under the curve from time 0 to the last time measured; C_{max} = maximum plasma concentration; EC = enteric-coated; LS = least-squares; PL2200 = a modified-release lipid-based aspirin; T_{max} = time until maximum acetylsalicylic acid concentration.

formation over baseline (20,21). These are patients who may be considered intrinsically aspirin resistant.

Extent of inhibition of ex vivo platelet function. The functional impact of the differences in COX-1 inhibition, as evidenced by the differences in serum TXB_2 with the 3 aspirin formulations, was reaffirmed by using platelet light transmittance aggregometry ex vivo. As expected, the kinetics of TXB_2 depletion and nonresponsiveness seemed to be consistent with AA-induced platelet aggregation (Figure 3). Unlike plain aspirin and PL2200, EC aspirin maximum inhibition of AA-induced aggregation was observed after multiple doses. However, the collagen response was more variable (data not shown).

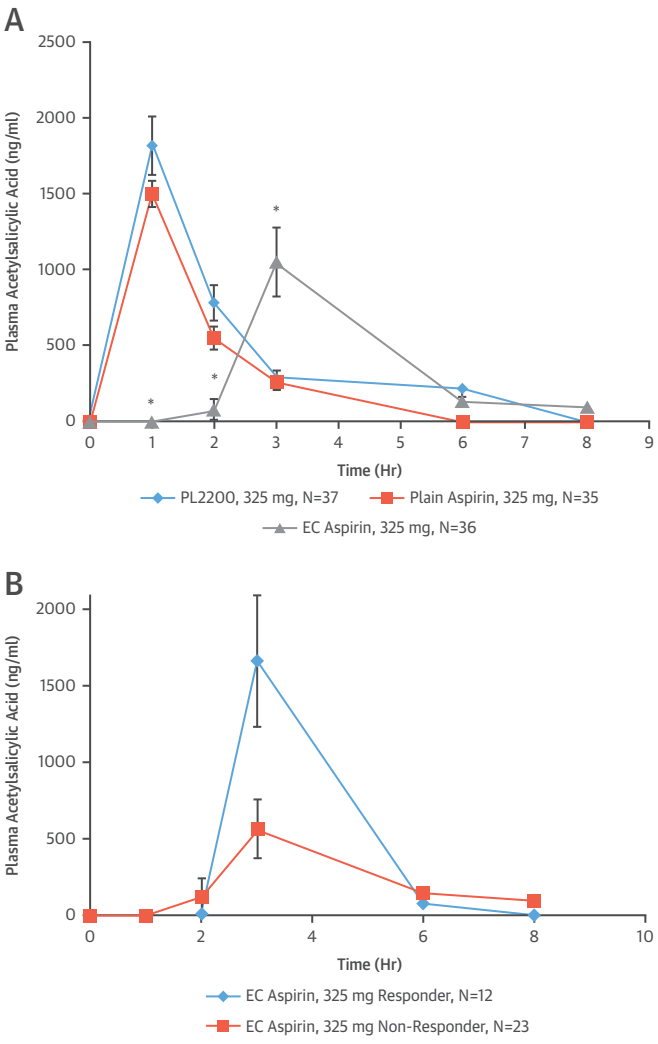
Aspirin nonresponsiveness may be due to poor aspirin absorption. We directly measured the plasma concentrations of aspirin at the same time as serum TXB_2 and platelet aggregation after the initial doses of the study drugs. The functional response was consistent with the single-dose bioavailability of ASA. C_{max} and AUC_{0-t} were significantly lower, and the time to reach C_{max} was significantly higher, with EC aspirin compared with either plain aspirin or PL2200 (Table 2, Figure 4A). The C_{max} and AUC_{0-t} values for PL2200 were approximately 2.3- and 4.5-fold higher than those for EC aspirin ($p < 0.0001$) but similar to those for plain aspirin. Regression analysis indicated that the lower rate and extent of absorption for EC aspirin were consistent with a lower extent of COX-1 inhibition with marked intraindividual variability (data not shown).

If the high rate of incomplete responsiveness on the basis of TXB_2 is indeed mediated by a lower extent of absorption, the ASA C_{max} for EC aspirin-treated responders should be greater than that for nonresponders. Therefore, in a post hoc analysis, each patient was classified as an EC aspirin responder or nonresponder on the basis of the single-dose response criterion of ≥99% depletion of TXB_2. Among the EC aspirin group, incomplete responders, as assessed by using TXB_2 levels, had significantly lower C_{max} and more often had an ASA concentration <150 ng/ml at all time points (Table 3, Figure 4B). These findings suggest that an incomplete response to EC aspirin is mediated by reduced absorption of ASA.

OFFSET OF ANTIPLATELET ACTIVITY. Offset of antiplatelet activity was evaluated by assessing repletion of platelet COX-1 activity after the last dose. Regeneration rates of COX-1 activity were similar for all 3 study drugs, with least squares mean ± SE of 1.54 ± 0.05, 1.57 ± 0.05, and 1.61 ± 0.05 ng TXB_2 /ml/h for PL2200, plain aspirin, and EC aspirin, respectively.

Sequence effect. Treatment sequence was included in the mixed effects models. The p values in all of the

FIGURE 4 Plasma ASA Concentration Versus Time Profiles



(A) Plasma acetylsalicylic acid (ASA) concentration versus time profiles after administration of PL2200, plain aspirin, and EC aspirin. The extent of aspirin bioavailability after EC aspirin was lower. ASA concentration (mean ± SE) is illustrated. **Asterisks** denote time points at which ASA levels associated with EC aspirin were significantly different ($p < 0.0001$) from those after dosing of PL2200 and plain aspirin. The p values are from 2-sample Student *t* tests. **(B)** Extent of aspirin absorption determines aspirin responsiveness. Plasma ASA concentration versus time profiles after EC aspirin administration for aspirin responders and nonresponders. An aspirin responder is defined as a patient whose TXB_2 inhibition level reached ≥99.0% within 24 h after the first dose. Mean observed ASA maximum plasma concentration (C_{max}) (mean ± SD) for responders (1,658.6 ± 1,355.3 ng/ml) is significantly different ($p = 0.027$) from that for nonresponders (670.3 ± 882.3 ng/ml). N = number of aspirin responders or nonresponders with a quantifiable ASA C_{max} and therefore included in this analysis; p value from chi-square test and 2-sample Student *t* test for categorical and continuous variables, respectively. Abbreviations as in Figure 1.

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TABLE 3 Comparison of Aspirin C_{max} in EC-Treated Responders and Nonresponders

	Responders	Nonresponders	p Value*
C_{max}, ng/ml ASA	1,658.58 ± 1,355.26 (10)	670.31 ± 882.30 (18)	0.027
C_{max} ASA concentration <150 ng/ml	16.67 (2/12)	52.17 (12/23)	0.042

Values are mean ± SD (N) or % (n/N). *The p values were assessed by using the 2-sample Student *t* test for continuous variables and the chi-square test for categorical variables. The plasma 150-ng/ml ASA cutoff is on the basis of plasma values 3 times the lower limit of quantitation of the method. Nonresponders were defined as those with ≤99% inhibition of thromboxane B_2.

ASA = acetylsalicylic acid; other abbreviations as in Table 2.

mixed effects models in all the PD and PK analyses were >0.4, indicating that the washout interval was effective and the order of study drug treatments had no effect.

Multivariable analysis. In a post hoc multivariable analysis, female sex was a significant independent predictor of nonresponsiveness to EC aspirin ($p = 0.0414$). Age, race, body mass index, duration of diabetes, or glycemic control were not independent predictors.

DISCUSSION

The results of this PK/PD trial show that there is substantial variability in antiplatelet response to dosing with 325 mg of EC aspirin in patients with diabetes mellitus. In fact, a significant proportion of patients receiving EC aspirin meeting the criteria for nonresponders had no detectable plasma ASA level at the times measured. When crossed over to plain aspirin or PL2200, significantly greater ASA absorption, COX-1 inhibition, and rate of responders were observed. Only 2 subjects were nonresponders to all 3 drug formulations, indicating that the incidence of intrinsic, nonformulation-dependent resistance to aspirin is very low. As such, among the various mechanisms of aspirin resistance invoked, the simplest explanation for reduced aspirin responsiveness may be due to reduced formulation-dependent bioavailability of ASA.

These results confirm that if a rapid aspirin effect is necessary, as in acute myocardial infarction, an immediate-release formulation (i.e., plain aspirin) is the therapy of choice. For chronic dosing as well, there does not seem to be any benefit with EC aspirin. Previous EC aspirin data did not show a beneficial effect on gastrointestinal bleeding (13), and the present study suggests that there is a 2- to 3-day period of diminished antiplatelet response after initial dosing versus use of plain aspirin or a lipid-based formulation of aspirin. Whether this scenario confers actual cardiovascular risk requires further evaluation in clinical trials.

A prior endoscopic study noted a particularly high rate of endoscopic ulceration with plain aspirin (14). PL2200 may potentially reduce gastrointestinal complications compared with plain aspirin but without the variability in antiplatelet response noted with EC aspirin. This hypothesis would need to be tested in a chronic therapy trial adequately powered for clinical events.

The results with respect to TXB_2 levels and platelet aggregation response to AA were consistent in the present study. Further studies with several different agonists will be necessary to more precisely discriminate the antiplatelet effect among aspirin formulations. However, TXB_2 levels and response to AA to assess the inhibitory effects of aspirin's primary biochemical target, COX-1, are the most widely used approaches to gauge the effect of aspirin on platelets.

STRENGTHS AND LIMITATIONS. Strengths of this study include that it systematically evaluated aspirin absorption, COX-1 inhibition, and platelet function, while controlling for potential confounders of aspirin nonresponsiveness, including aspirin compliance, standardization of timing of blood sampling, and timing of administration of other drugs and food in a crossover design. To the best of our knowledge, this study is the largest PK and PD evaluation of aspirin in patients with diabetes mellitus.

Limitations of this study include the 3-day duration. It is possible that with more chronic dosing of EC aspirin there would have been less variability in antiplatelet effect. Nevertheless, the degree of aspirin nonresponsiveness seen with EC aspirin in the first 3 days after initiation may have clinical importance in patients with chronic cardiovascular disease starting aspirin after a period of discontinuation; these scenarios would include for an invasive procedure or because of periodic nonadherence, or in patients with heightened platelet reactivity, such as those with acute coronary syndromes or stroke. Also, because only obese patients with diabetes were studied, further research will be necessary to see if findings in nondiabetic patients with indications for aspirin therapy would be similar; in healthy volunteers, regular daily dosing of EC aspirin under controlled conditions resulted in stable platelet inhibition (12,23).

For the purposes of safety, this crossover study enrolled patients with diabetes without established cardiovascular disease; thus, it is not possible to comment on what would have happened in diabetic patients with cardiovascular disease. One might presume that any variability with the enteric coating noted in this study would be further exacerbated and the potential clinical implications increased in those with atherosclerosis or activated platelet levels.

In addition, because 325 mg of aspirin was used in each arm of the present study, it is unknown what would have happened with aspirin 81 mg. However, given that variability with EC aspirin was high with 325 mg, it might be even higher with lower aspirin doses, as observed in previous research with 81 mg of daily EC aspirin, and the time required to achieve a therapeutic level of inhibition would likely be much longer (24). Importantly, aspirin resistance remains difficult to define unequivocally, and our study design cannot provide data on cardiovascular outcomes or other potential clinical implications associated with aspirin use in patients with diabetes (25). Finally, these results pertain to effects of enteric coating on aspirin absorption. Whether an enteric coating decreases, increases, or does not affect the absorption of other important drugs depends on an array of factors, such as the site of preferential drug absorption, the polarity of the drug, and the chemical composition of the coating.

CONCLUSIONS

In patients with diabetes, the use of EC aspirin was associated with greater nonresponsiveness due to reduced bioavailability than either regular plain aspirin or a lipid-based formulation of aspirin. Although the clinical implications need to be established, this finding does raise potential concerns that patients taking EC aspirin may not receive the full cardioprotective benefit of this important drug.

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ADDRESS FOR CORRESPONDENCE: Dr. Deepak L. Bhatt, Brigham and Women's Hospital Heart and Vascular Center, Harvard Medical School, 75 Francis Street, Boston, Massachusetts 02115. E-mail: dlbhattmd@post.harvard.edu.

PERSPECTIVES

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS: EC aspirin is associated with a lower antiplatelet response due to impaired gastrointestinal absorption.

TRANSLATIONAL OUTLOOK: Further studies are needed to assess the clinical relevance of this PK and PD observation.

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KEY WORDS acetylsalicylic acid, enteric coating, pharmacodynamics, pharmacokinetics, platelet function, thromboxane

APPENDIX For a supplemental figure, please see the online version of this article.