Drug Resistance and Pseudoresistance
An Unintended Consequence of Enteric Coating Aspirin

Tilo Grosser, MD*; Susanne Fries, MD*; John A. Lawson, MS; Shiv C. Kapoor, PhD; Gregory R. Grant, PhD; Garret A. FitzGerald, MD

Background—Low dose aspirin reduces the secondary incidence of myocardial infarction and stroke. Drug resistance to aspirin might result in treatment failure. Despite this concern, no clear definition of aspirin resistance has emerged, and estimates of its incidence have varied remarkably. We aimed to determine the commonality of a mechanistically consistent, stable, and specific phenotype of true pharmacological resistance to aspirin—such as might be explained by genetic causes.

Methods and Results—Healthy volunteers (n=400) were screened for their response to a single oral dose of 325-mg immediate release or enteric coated aspirin. Response parameters reflected the activity of the molecular target of aspirin, cyclooxygenase-1. Individuals who appeared aspirin resistant on 1 occasion underwent repeat testing, and if still resistant were exposed to low-dose enteric coated aspirin (81 mg) and clopidogrel (75 mg) for 1 week each. Variable absorption caused a high frequency of apparent resistance to a single dose of 325-mg enteric coated aspirin (up to 49%) but not to immediate release aspirin (0%). All individuals responded to aspirin on repeated exposure, extension of the postdosing interval, or addition of aspirin to their platelets ex vivo.

Conclusions—Pharmacological resistance to aspirin is rare; this study failed to identify a single case of true drug resistance. Pseudoresistance, reflecting delayed and reduced drug absorption, complicates enteric coated but not immediate release aspirin administration.


Key Words: aspirin ■ blood platelets ■ pharmacology ■ thromboxanes

The efficacy of low-dose aspirin in the secondary prevention of important vascular events, such as stroke and myocardial infarction, is well established, whereas its relative benefit versus the risk of gastrointestinal bleeding in primary prevention is still debated.1–4 The mechanism by which this benefit is accrued is explained sufficiently by the irreversible acetylation of Ser530 in the enzyme prostaglandin G/H synthase-1 (commonly termed cyclooxygenase [COX]-1) and the consequent suppression of thromboxane (Tx) A1 (TXA1) formation.3,5 TXA1 is synthesized from arachidonic acid in activated platelets and released as a local signal, which amplifies activation and recruits additional platelets to the site of clot formation.6,7 However, TXA1 is but one of several endogenous agonists that result in platelet activation and vasoconstriction, both pertinent to clinically important vascular occlusive events.3,7 Thus, inhibition of TXA1 signaling substantially limits the aggregation response, but it does not completely prevent clot formation. Given this capacity for redundancy, the magnitude of the clinical impact of aspirin is perhaps surprising; for example, it reduces both death and myocardial infarction by 50% in placebo-controlled trials of patients with unstable angina.8,9 What is unsurprising is that treatment failures occur on aspirin, as they do in patients taking other cardiovascular drugs. Many reasons for this have been invoked, from patients failing to take their medication to aspirin-insensitive mechanisms of platelet activation to drug–drug interactions.10–14 Despite this, the concept of aspirin resistance has emerged,15 prompting the development of point-of-care diagnostic approaches to assessing the inhibition of platelet aggregation in patients taking aspirin.16 Estimates of the incidence of such a resistant phenotype have varied widely,16–20 and the reliance on an assay of platelet aggregability as a surrogate for enzyme acetylation and suppression of platelet TXA2 formation21 and for clinical outcomes15 has been criticized. Similarly, reliance on post hoc analysis of the major urinary Tx metabolites22 as a biomarker of resistance in large scale trials23,24 has been questioned.15

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True drug resistance to aspirin would result from (1) a failure to reach its molecular target, (2) a failure to acetylate COX-1 despite sufficient concentrations in the platelet, or (3) a failure...
of COX-1 acetylation to suppress thromboxane formation. In the present study, we sought to parse the variance in response to aspirin and to estimate the incidence of an internally consistent phenotype of true drug resistance, reconciling biochemical and functional assays of aspirin action that are stable over time and specific for the effect of aspirin on platelets. Healthy volunteers (n=400) were screened for their response to a single oral dose of 325 mg regular immediate release aspirin or enteric coated aspirin, which was designed to reduce local damage of the gastric mucosa. Platelet aggregation induced by arachidonic acid, serum thromboxane formation, and urinary excretion of a Tx metabolite (TxM)—all reflecting the activity of the molecular target of aspirin, COX-1—were measured before and after dosing. Individuals who appeared to be aspirin resistant underwent repeat testing. Individuals who failed to respond to aspirin twice were exposed to low-dose enteric coated aspirin (81 mg) and clopidogrel (75 mg) for 1 week each in a cross over design.

Although the distinct estimates of aspirin action were congruent and apparent resistance based on platelet aggregation inhibition at a single time point was common, this reflected variable drug exposure attributable to enteric coating of aspirin and was either inconstant over time or could be overcome by addition of aspirin ex vivo. Thus, we failed to find a single case of true drug resistance in this study of 400 healthy volunteers.

Methods

Study Design

The study protocol was approved by the Institutional Review Board of the University of Pennsylvania and conducted in University’s Clinical Translational Research Center (CTRC). Healthy, nonsmoking volunteers (aged 18–55 years) who provided written informed consent were enrolled and abstained from all medications and nutritional supplements for the duration of the study.

In Phase 1, fasted healthy volunteers (n=400) received a single oral dose of 325 mg aspirin, and drug response parameters were assessed before and after dosing. Two distinct aspirin formulations were administered with 250 mL water, followed by a light standardized meal 2 hours later. Drug responsiveness was assessed at 4 or 8 hours after administration to create 3 groups (Figure 1). Group 1 (n=40) received regular, immediate release aspirin (Genuine Bayer Aspirin, Bayer Health Care, Morristown, NJ), and their response was assessed 8 hours after dosing. Group 2 (n=210) received enteric coated aspirin (Safety Coated Bayer Aspirin), and their response was measured 8 hours after dosing. Group 3 (n=150) received enteric coated aspirin, and their response was assessed at 4 hours. Enteric coating retards aspirin absorption markedly and renders this process much more variable.25,26 Thus, Groups 2 and 3 were characterized by decreasingly stringent conditions for the identification of variation in the response to aspirin. This would be expected to increase the likelihood of identifying host factors (eg, genetics, nutritional habits, the composition of the gut microbiome) that might influence reproducibly the response to aspirin. Decisions regarding inclusion of subjects into the next study phases were made based on a point-of-care assessment, the platelet aggregation response to arachidonic acid, which was performed immediately after the blood draw. Individuals who showed <60% inhibition of their maximal arachidonic acid induced platelet aggregation (comparing post versus predose aggregation) were classified as nonresponders, who might potentially be resistant to aspirin and entered Phase 2 of the study. Additionally, a subset of individuals who showed an adequate inhibition of ≥60% was invited to participate in Phase 2 as responder controls.

Phase 2 addressed the stability of the phenotype of aspirin resistance. After a washout period of at least 14 days, the 2 cohorts identified in Phase 1 (108 nonresponders and 149 responders) underwent repeat testing with a single dose of aspirin. Again, individuals showing <60% inhibition of platelet aggregation using arachidonic acid as a stimulus were classified as nonresponders. Those who were nonresponders in Phase 1 and Phase 2 were invited to proceed to Phase 3. They were matched by gender, age (±2.5 years) and race with subjects who were responders in Phase 1 and Phase 2.

Phase 3 determined whether resistance persisted when the dose of aspirin was altered and multiple doses were administered. It also assessed whether the phenotype was specific for aspirin or extended to another platelet inhibitor, clopidogrel. Two cohorts (27 nonresponders and 25 responders, who completed the study) received 81-mg enteric coated aspirin and 75-mg clopidogrel for 7 days, in a

Figure 1. Study design for Phase 1 and 2 (A) and Phase 3 (B).
crossover design randomized by order and separated by a washout period of at least 14 days (Figure 1). Fifteen of the 42 volunteers, who were classified nonresponders in both Phase 1 and Phase 2, were unavailable for retesting in Phase 3 mostly as a result of geographical mobility. However, the nonresponder cohort included all individuals who had appeared resistant in Phase 2 even when aspirin was added to their platelets ex vivo. Outcome measurements were obtained before treatment began, 4 hours after the first dose, just before the last dose and 4 hours after the last dose. Aspirin response status was assessed based on the last inhibition detected in the last measurement relative to baseline. Those who were responsive to clopidogrel but showed an impaired response only in the COX-1 pathway were determined by arachidonic acid induced platelet aggregation and ex vivo whole blood TxB2 release in all 3 study phases were considered pharmacologically aspirin resistant.

Efficacy Assessments
These included (1) platelet function tests ex vivo involving traditional optical platelet aggregation testing of 4 pathways of platelet activation (500 µg/mL arachidonic acid, 20 µmol/L adenosine diphosphate (ADP), 2 µg/mL collagen, 100 µmol/L the protease receptor-1 activating peptide [PAR-1AP]).13 The sensitivity of platelets to aspirin ex vivo was determined by addition of freshly prepared aspirin (dissolved in ethanol) at final concentrations of 30 and 100 µmol/L to PRP, 15 minutes before platelets were stimulated. (2) The capacity of platelets to form Tx during clotting was assessed as an index of COX-1 enzymatic activity ex vivo. Serum TxB2 was detected by enzyme immunoassay (Cayman Chemicals, Ann Arbor, MI).21 (3) The urinary excretion of the Tx metabolite, 11-dehydro TxB2 (TxA)—an index of platelet COX-1 activity in vivo—was quantified as its methoxyamine derivative by liquid chromatography tandem mass spectrometry in spot urine samples that were collected 30 minutes after voiding.28

Statistical Considerations
Empirical cumulative frequency distributions were graphed in R (cran.r-project.org). The effect of aspirin exposure on the outcome variables (post versus predose comparison) was assessed with the Wilcoxon Matched-Pairs Signed Ranks Test as implemented in R. A P<0.05 was considered statistically significant.

Responder status to aspirin in the arachidonic acid induced aggregation and the serum TxB2 assays was reported using thresholds that were derived from data sets generated previously in our laboratory.13,21 Inhibition of aggregation and TxB2 formation relative to baseline (before aspirin administration) was calculated. Failure of aspirin to inhibit arachidonic acid-induced aggregation >60% or serum TxB2 formation >95% was classified as nonresponse. These threshold values were chosen before the study was conducted. Responders/nonresponders did not exhibit any differences in baseline AA aggregation measures before the administration of aspirin.

For all other outcome measures (maximal aggregation, maximal slope of aggregation, absolute serum TxB2 concentration, urinary TxB metabolism), a data driven classification threshold for responder versus nonresponder status was identified using a modified Receiver Operating Characteristics (ROC) analyses with the inhibition of serum TxB2 formation as the gold standard. Thus, all values of a given outcome measure (eg, maximal arachidonic acid induced aggregation) were considered as hypothetical cutoffs to predict class membership. For each hypothetical cutoff all instances of an outcome measurement were assigned a hypothetical class label. However, instead of assigning the true class labels based on an arbitrary threshold of serum TxB2 inhibition we allowed the best fitting random forest model19 of serum TxB2 inhibition to define true class membership. The random forest classifier as implemented in the R package randomForest was run in classification mode for each hypothetical threshold. Each random forest model, consisting of 4000 trees, generated a confusion matrix with the classes predicted by the hypothetical cutoff and the true classes derived from the random forest fit of the serum TxB2 inhibition distribution. Graphs of true positive and true negative rates were used to evaluate the performance of the hypothetical thresholds. The random forest algorithm also generates an error estimate of the model fit, which is the error rate we report here together with the classification accuracy.

Results
Parsing Variability in the Response to Aspirin
We studied a population of relatively young and healthy individuals (Table 1) to assess the inter- and intradividual variability in the pharmacological response to aspirin without environmental variability contributed by disease. High inter- and low intradividual variability would suggest that host factors which predict the response to aspirin might be identified even in a much more heterogeneous population with cardiovascular disease. All 40 subjects administered immediate release aspirin (Group 1) in Phase 1 responded with a reduction of platelet aggregation induced by direct stimulation of the COX-1 pathway by arachidonic acid by >60% comparing pre- and postdose measurements (Table 2). By contrast, 17% qualified as nonresponders 8 hours after dosing with enteric coated aspirin (Group 2), increasing to 49% 4 hours after the dose (Group 3).

If platelets are not inhibited by ingestion of aspirin, but can be inhibited by addition of aspirin ex vivo, this points to a pharmacokinetic rather than a pharmacodynamic explanation for such a nonresponder status. The addition of aspirin ex vivo dose dependently corrected nonresponder status in the 2 groups of volunteers administered enteric coated aspirin, falling from 17% to 0% 8 hours (Group 2) and from 49% to 12% 4 hours after dosing (Group 3) (Table 2). Assessment of the response to aspirin by quantitation of serum TxB2, which is considered a reliable biochemical measure of platelet COX-1 function, but is not practical as a

<table>
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<th>Group</th>
<th>Aspirin formulation</th>
<th>Assessment time</th>
<th>Ethnicity/race, n</th>
<th>Sex (%)</th>
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<td>American Indian or Alaskan Native</td>
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<tr>
<td>Group 2</td>
<td>EC</td>
<td>8 h</td>
<td>Asian</td>
<td>2</td>
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<td>Group 3</td>
<td>EC</td>
<td>4 h</td>
<td>Native Hawaiian or Pacific Islander</td>
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</tr>
<tr>
<td>Total</td>
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<td>Black (Non-Hispanic)</td>
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<td>Median</td>
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<td>21.1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3. Quartile</td>
<td>28.8</td>
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Table 2. Nonresponse to Administration of 325-mg Aspirin in Study Phases 1 and 2 as Assessed by Arachidonic Acid Induced Platelet Aggregation

<table>
<thead>
<tr>
<th>Status</th>
<th>Group 1-Plain Aspirin, 8 h Postdrug</th>
<th>Group 2-EC Aspirin, 8 h Postdrug</th>
<th>Group 3-EC Aspirin, 4 h Postdrug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresponders in Phase 1</td>
<td>0 (0/40)</td>
<td>17 (35/210)</td>
<td>49 (73/150)</td>
</tr>
<tr>
<td>Nonresponders in Phase 1 AND 2</td>
<td>0 (0/40)</td>
<td>6 (12/209)</td>
<td>20 (30/150)</td>
</tr>
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Regular or enteric coated (EC) aspirin was administered and the response assessed either 8 or 4 hours postdosing. Platelet function was also measured in platelet-rich plasma that was treated with 100 micromolar aspirin ex vivo for 15 minutes. Nonresponders were arbitrarily defined as individuals who had an inhibition of platelet aggregation induced by arachidonic acid of <60% when comparing with predose assessments.

Table 3. Nonresponse to Administration of 325-mg Aspirin in Study Phases 1 and 2 as Assessed by Serum TxB₂

<table>
<thead>
<tr>
<th>Status</th>
<th>Group 1 Plain Aspirin, 8 h Postdrug</th>
<th>Group 2 EC Aspirin, 8 h Postdrug</th>
<th>Group 3 EC Aspirin, 4 h Postdrug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresponders in Phase 1</td>
<td>3 (1/40)</td>
<td>24 (35/210)</td>
<td>59 (88/150)</td>
</tr>
<tr>
<td>Nonresponders in Phase 1 AND 2</td>
<td>0 (0/40)</td>
<td>7 (14/199)</td>
<td>26 (39/146)</td>
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Regular or enteric coated (EC) aspirin was administered and the response assessed either 8 or 4 hours postdosing. Nonresponders were arbitrarily defined as individuals who had <95% inhibition of serum TxB₂, when comparing post- and predosing assessments. TxB₂ indicates thromboxane B₂.

Diagnosis of an Individual’s Aspirin Response Status
The study sought individuals who showed an inadequate mechanistic response to the administration of aspirin as assessed whether the phenotype was specific for aspirin or extended to another platelet inhibitor, clopidogrel, which targets an ADP receptor (the P2Y₁₂). Twenty-seven individuals who were nonresponders and 25 who were responders based on arachidonic-induced platelet aggregation in both Phase 1 and Phase 2 received 81-mg aspirin for 7 days and 75-mg clopidogrel for 7 days, in a crossover design (Figure 1). Only 1 volunteer in the nonresponder cohort failed to respond to the week-long treatment with aspirin in Phase 3 as assessed by arachidonic acid–induced platelet aggregation and serum TxB₂ inhibition (Figure 2). However, platelets from this subject responded to aspirin addition ex vivo. Furthermore, this apparent resistance was not specific as this individual also failed to respond to clopidogrel (not shown). Interestingly, 2 individuals who entered Phase 3 as controls with demonstrated ability of their platelets to respond to aspirin administration in Phase 1 and 2 now failed to respond in Phase 3 (Figure 2). One of them also failed to respond to clopidogrel (not shown), but both responded to addition of aspirin ex vivo.
assessed by platelet aggregation ex vivo, serum TxB₂ formation—an index of the capacity of platelets to form COX-1 dependent TXA₂—and the urinary excretion of the thromboxane metabolite, 11-dehydro TXB₂ (TxM)—an index of actual thromboxane biosynthesis. All 3 measurements detected a significant difference between the pre- and postdose measurement in each treatment group (P<0.01, data not shown). Plotting their relative cumulative frequency distributions illustrates the utility of the drug response markers to determine response status at the individual level (Figure 3). The distribution curves for arachidonic acid induced platelet aggregation and serum TxB₂ concentration, but not for urinary TxM excretion, allowed the data driven detection of a threshold value—a plateau in the distribution curve—which distinguishes between responders and nonresponders. A threshold of 16% (95% confidence interval 12% to 62%) residual arachidonic acid–induced platelet aggregation resulted in a test accuracy of >90% and an error rate of 10%. Indeed, the extended plateau in the frequency distribution renders arachidonic acid–induced platelet aggregation essentially a dichotomous outcome measurement (see, for example, the postdose distribution of Group 3, the group with ≈50% apparent resistance, in Figure 3C). Stimuli of platelet aggregation that activate platelet pathways in which COX-1–dependent events are downstream of the initial stimulus—collagen and ADP—were much less reliable diagnostic tools for the assessment of the response status (Figure 4). They resulted in accuracies of 80% and 75% and error rates of 21% and 36%. As expected, platelet activation with proteinase activated receptor-1 activating peptide (PAR-1AP), which ligates a thrombin receptor, did not associate with inhibition of serum Tx formation by aspirin.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Relative cumulative frequency distributions of the arachidonic acid (325 mg)–induced platelet aggregation response (A–C), serum thromboxane formation (D–F), and urinary thromboxane metabolite (TxM) excretion (G–I) stratified by drug formulation and assessment time. The density of the data points is visualized by color coding—green indicates high density/frequency; red, low density/frequency. Classification thresholds (responders vs nonresponders) as determined iteratively using random forest classifiers are shown as blue arrows, which also depict the fraction of responders in the study groups. Urinary TxM excretion failed to predict individual response status, although pre- and postdose distributions segregated in each group (P<0.05).
Measurement of the absolute serum TxB₂ concentration (rather than serum TxB₂ inhibition based on the comparison of pre- and postdose concentrations) is an assessment that could be performed in patients without stopping aspirin therapy. Analysis of the frequency distribution of the absolute serum TxB₂ concentration also revealed a plateau that segregated the population into apparent responders and apparent nonresponders (Figure 3). A threshold of 29 ng/mL (95% confidence interval 26 ng/mL to 78 ng/mL) serum TxB₂ resulted in an accuracy of 98% and an error rate of 2%.

The congruence between arachidonic acid induced aggregation and serum TxB₂ formation for the diagnosis of nonresponse was high. One hundred five of 108 (97%) subjects classified as nonresponsive by arachidonic acid–induced platelet aggregation in Phase 1 also showed incomplete suppression of serum TxB₂ formation. However, measurement of serum TxB₂ identified 32 of 400 (8%) individuals as nonresponders who had not been recognized by arachidonic acid–induced platelet aggregation in Phase 1. Most of these had serum TxB₂ levels between 10 and 100 ng/mL, which represents a partially suppressed range when compared with median pre- and postdose concentrations of 315 ng/mL (interquartile range, 193–450 ng/mL) and 4 ng/mL (interquartile range, 2–133 ng/mL).

Analysis of the urinary TxM excretion data did not result in a threshold value that would allow for distinction of responders from nonresponders at the individual level with any reasonable accuracy (Figure 3).

**Discussion**

Aspirin blocks just 1 pathway of platelet activation, and treatment failures may result from the dominant role of other platelet agonists, combination of aspirin with other antiplatelet drugs, such as antagonists of the P2Y₁₂ receptor for ADP, sometimes confers a measurable incremental benefit. Other reasons for treatment failure include failure to consume the medication, accelerated platelet turnover, or interaction with reversible, competitive active site inhibitors of the COX-1 enzyme, such as ibuprofen or naproxen.

The term aspirin resistance is often used to imply treatment failure, although clinical outcomes have rarely been assessed—not unlike studies of the pharmacogenomics of clopidogrel. More commonly, a patient is classified as resistant to aspirin, if platelet aggregation, measured on a single occasion, is not depressed to an arbitrary degree. Estimates of the frequency of aspirin resistance have varied from 5% to 20% in most studies. Here we wished to determine the commonality of an internally consistent, specific phenotype of pharmacological resistance to aspirin that was stable over time, such as might result from gene variants in aspirin metabolizing enzymes or in the platelet COX-1-TxA₂ synthesis/
response network. This might be more readily discernible in a younger population without confounding disease variables and would be measurable by tests that reflected the mechanism of drug action.

The irreversible acetylation of platelet COX-1 is a sufficient mechanism to explain the cardioprotection afforded by aspirin, however direct assays have yet to be deployed to characterize resistance. The immediate consequence of drug action is suppression of formation of platelet TxA₂, quantitatively reflected by the analysis of its inactive hydrolysis product, TxB₂, in serum generated under controlled conditions. Measurement of serum TxB₂ reflects dose-dependent suppression by aspirin, cumulative inhibition during repeated daily dosing, and delayed recovery of platelet thromboxane formation after cessation of aspirin, driven by platelet turnover time. Urinary Tx metabolites also reflect the pharmacodynamic impact of aspirin on platelets and, indeed, derive largely from platelets under physiological conditions. However, other nonplatelet sources of Tx biosynthesis are also reflected by urinary metabolites and their contribution may increase under conditions of disease or physiological perturbation. Here, we found that large variability in urinary TxA₄ excretion renders it impracticable to assess even a healthy individual’s aspirin response status, although the reduction in mean urinary TxA₄ by aspirin was clearly detectable in a group of treated individuals. Finally, platelet aggregation responses induced by the COX enzyme substrate, arachidonic acid, depend on its transformation to TxA₄, and are suppressed by aspirin. Although other agonists aggregate platelets directly, secondary release of TxA₂ may amplify the response and result in partial inhibition of the aggregation signal by aspirin. A complication is that the relationship between suppression of platelet thromboxane and aggregation is very nonlinear—the capacity of platelets to generate thromboxane has to be suppressed by >95% before functional inhibition of the aggregation response is attained. This explains why diagnostic approaches which tested the COX pathway directly had the most favorable test sensitivities and false classification rates for identification of nonresponders.

We found that under acute dosing conditions, the frequency of resistance based solely on the failure to suppress the aggregation response to arachidonic acid was conditioned by the formulation of aspirin and the timing of the measurement. Resistance was measurable after enteric coated, but not immediate release formulations of aspirin, and appeared more frequent after the former when measurements were performed 4 hours rather than 8 hours after dosing. This pattern of variance was reflected both by the aggregation response to arachidonic acid and by serum TxB₂. By contrast, aspirin had minor effects on aggregation induced by ADP and collagen and no detectable effect on the activation of aggregation through the thrombin pathway by the protease receptor-1 activating peptide (PAR-1AP). The incidence of apparent resistance to enteric coated aspirin based on a single measurement of aggregation was 49% at 4 hours (Group 3) dropping to 17% at 8 hours (Group 2) after dosing in Phase 1 of the study. Strikingly, there was marked discordance between the individuals who seemed resistant on the 2 occasions; only in 20% at 4 hours and 6% at 8 hours was resistance stable over time. Consistent with the suggestion that apparent resistance was largely reflective of variable drug exposure in the patients receiving enteric coated aspirin, we corrected the phenotype in 101 of 108 cases by adding aspirin ex vivo in Phase 1 or 2, and all of the remaining 7 cases responded to adding aspirin ex vivo in Phase 3.

In Phase 3 of the study we compared cases who had remained resistant to inhibition of platelet aggregation by ingestion of aspirin in the first two phases of the study and matched them by gender and age with controls, then subjecting both groups to a weeklong administration of aspirin and clopidogrel in a cross over design. Because 15 of the original 400 subjects who fulfilled the inclusion criteria for Phase 3 were lost to follow-up, we may have underestimated the incidence of true biological resistance. However, of the 27 cases enrolled in this phase only one appeared now to be resistant to aspirin as assessed by arachidonic acid–induced aggregation, and this individual had responsiveness restored by addition of aspirin ex vivo. Although this was consistent with resistance as a result of limited drug exposure in vivo, as might be caused by genetic variants in aspirin metabolism, the effect was not specific to aspirin: the volunteer failed also to exhibit clopidogrel-induced inhibition of ADP-induced platelet aggregation. The unreliability of baring a diagnosis of aspirin resistance on a single measurement of platelet aggregation was further emphasized by 2 individuals who appeared resistant among the controls in Phase 3, having been responsive to aspirin in the 2 earlier phases of the study. Here, again, responsiveness to aspirin was apparent by addition of the drug ex vivo and the subject was also unresponsive to clopidogrel. Thus, such resistance may have reflected a failure to ingest aspirin.

In summary, we performed studies in 400 healthy volunteers seeking to determine the commonality of a mechanistically consistent, temporally stable, and specific phenotype of true aspirin resistance, such as might be attributable to genomic variation in aspirin metabolism or in the platelet COX-1/TxA₂ synthesis response pathway. We failed to find a single person who satisfied these criteria. By contrast, pseudoresistance, resulting from delayed and reduced drug absorption, was common after ingestion of enteric coated aspirin. These observations question the value of seeking to diagnose aspirin resistance with single point-of-care diagnostic approaches and support the finding of inconsistent platelet inhibition after enteric coated preparations of aspirin.

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Disclosures
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References

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**CLINICAL PERSPECTIVE**

Low-dose aspirin reduces the secondary incidence of and mortality from stroke and myocardial infarction by the irreversible inhibition of the platelet cyclooxygenase/thromboxane A2 pathway. The term aspirin resistance is often used to imply treatment failure, although clinical outcomes have rarely been assessed. More commonly, a patient is classified as resistant to aspirin if platelet aggregation, measured on a single occasion, is not depressed to an arbitrary degree. Estimates of the frequency of aspirin resistance have varied widely. This study determined the incidence of true pharmacological resistance to aspirin—internally consistent functional and biochemical estimates of the inhibition of the cyclooxygenase/thromboxane A2 pathway, stability over time, and drug specificity—in the general population. Not a single case of true drug resistance such as might result from gene variants in the aspirin metabolizing or target pathway was found in 400 volunteers. However, test doses of enteric coated aspirin, but not regular immediate release aspirin, were associated with variable absorption, which would have been inaccurately interpreted as resistance by a single point-of-care test. Measurement of a urinary thromboxane metabolite, an U.S. Food and Drug Administration–approved approach to determining aspirin response status, was found unsuitable for diagnosing aspirin resistance in an individual, although it reflects inhibition of the aspirin target pathway in a population.
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