Aspirin Resistance: A Clinical Review Focused on the Most Common Cause, Noncompliance

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Abstract

Aspirin is an inexpensive, readily available medication that reduces the risk of subsequent vascular disease by about 25% in patients with known occlusive vascular disease. Aspirin's beneficial effect is mediated via inhibition of arachidonic acid (AA) activation of platelets and is detected by demonstrating a decrease in platelet function and/or a decrease in prostaglandin metabolites. Patients who are assumed to be taking their aspirin, but who do not demonstrate an aspirin effect are labeled as, "aspirin resistant." This is an unfortunate designation as the vast majority of patients labeled as "aspirin resistant" are noncompliant. Noncompliance is demonstrated in multiple studies that use repeat testing for platelet inhibition in patients with an initial inadequate response to aspirin. When the test is repeated under condition where ingestion of the test aspirin is assured, the patients' platelets are inhibited. Instead of using the term "aspirin resistance," this review will use "inadequate response to aspirin." Patients with an inadequate aspirin response have an increased likelihood for subsequent vascular events. Detection and treatment of an inadequate aspirin response would be facilitated by the development of a bedside assay that uses whole blood, is technically simple, inexpensive, sensitive, specific, reproducible, and provides an answer in a few minutes. Future research in patients with an inadequate response to aspirin should focus on mechanisms to improve compliance, which should decrease their risk of future vascular events.

Keywords

intracranial arteriosclerosis, aspirin, platelets

Introduction

Individuals with cardiac or cerebral vascular disease can decrease their risk of subsequent vascular events by 25%, if they take 1 aspirin daily.¹ The beneficial effect of aspirin is via inhibition of platelet function.² However, patients prescribed aspirin for cardiovascular diseases, who demonstrate minimal inhibition of platelet function by aspirin have an increased risk of a subsequent vascular event. Two meta-analyses demonstrate a higher probability (odds ratio 3.8) for a future major vascular event in these individuals.^{3,4} Approximately one quarter of patients with cardiovascular disease who are prescribed aspirin appear to have an inadequate response to aspirin. Inadequate response to aspirin results in a poorer prognosis, and these individuals have been labeled as "aspirin resistant." The term aspirin resistance is commonly used in the literature to delineate a group of patients whose platelets are weakly inhibited by aspirin using a specific technique. Aspirin resistance can be defined as "... suboptimum aspirin-induced inhibition of platelet function that has been proven to be an independent risk factor for an increased risk of a future vascular event."⁵ However, aspirin resistance is a misnomer because most frequently it is caused by failure

to take the aspirin. Furthermore, the term implies that a mechanistic reason limits aspirin's effectiveness. However, a biological explanation for an inadequate response to aspirin has not been demonstrated, leaving one to conclude that noncompliance is the primary reason for lack of aspirin's effect on platelets.^{5,6} Instead of perpetuating the term, "aspirin resistance," this review will substitute the phrase "inadequate aspirin response" to prescribed aspirin.

The focus of this review is the practitioner caring for a patient with progressive vaso-occlusive disease and whose platelet inhibition from prescribed aspirin appears to be inadequate.

Incidence and Significance

The incidence of an inadequate platelet response to prescribed aspirin, as reported in the review by Zimmerman, is highly

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variable and depends on the patients' condition. In patients with stable coronary arterial disease, reported incidence varies from 5% to 69%, which compares to an incidence of 22% to 83% in patients with an acute myocardial infarction (MI). In all, 20% to 74% of individuals studied immediately following coronary arterial bypass grafting were reported to have an inadequate response.⁷ We studied stable post-MI patients prescribed a daily aspirin for at least 1 month. Repeat testing in the 17 (9%) patients who initially demonstrated an inadequate response to prescribed aspirin showed inhibition of platelets in 16 of the 17 participants, supporting the notion that noncompliance is the primary cause of "aspirin resistance."⁸ A recent review suggests that a reasonable estimate of laboratory-confirmed inadequate platelet response to prescribed aspirin is approximately 25%.⁹ This review supports the thesis that the majority of these individuals are noncompliant.

When patients whose inadequate response to aspirin is detected using a variety of methods are compared with aspirin-sensitive patients, they have an increased probability for a subsequent significant vascular event that is usually defined using a composite outcome that includes cardiac and cerebral vascular events and vascular death. In all, 9% of post-MI patients who admitted not taking their prescribed aspirin died, which compares with the death of only 3% of those who continued taking their aspirin.¹⁰ Similar results were obtained in a study of patients with cerebral vascular disease. Thirty percent of patients with recurrent cerebral vascular events had an inadequate platelet response to prescribed aspirin; all individuals who did not have recurrent cerebral vascular events demonstrated aspirin-induced platelet inhibition.¹¹ Poststroke patients were evaluated 12 hours after 500 mg of prescribed aspirin. Patients whose platelets were not inhibited had a subsequent vascular event rate of 40%, while those whose platelets were inhibited by aspirin had only a 4% rate.¹² In a group of patients with stable coronary arterial disease, the "Verify-Now" point-of-care assay identified 27% of patients whose platelets were not inhibited by aspirin and the risk of a subsequent vascular event in this group was significantly increased (hazard ratio 3.1) when compared to individuals whose platelets were inhibited by aspirin.¹³ The effect of an inadequate response to aspirin on subsequent cardiovascular events was evaluated in 326 patients prescribed aspirin for stable cardiovascular disease. Platelet response was evaluated using optical aggregometry with the agonist adenosine diphosphate (ADP) and arachidonic acid (AA; Figure 1). Major cardiovascular events occurred in 34 (10%) of 326 patients. In all, 17 patients were classified as having an inadequate platelet response to prescribed aspirin and 24% (4 of these 17) had a subsequent vascular event, while major cardiovascular events occurred in a smaller percentage 10% (30 of 309) patients with an adequate platelet response to aspirin. Although the number of inadequate aspirin response patients is small, this pioneering study did suggest that those patients classified with an inadequate aspirin response were at an increased risk of a subsequent vascular event.¹⁴ In the above studies, those patients

Figure 1. Light aggregation: as platelets in platelet-rich plasma are stimulated to aggregate, increased light is transmitted through the cuvette. A light sensor connected to a graphic readout allows for measurement of the time for aggregation to begin, the rate of platelet aggregation, and the percentage of full aggregation observed.

judged to have an inadequate aspirin response did not have a repeat evaluation under conditions that ensured compliance.

Two meta-analyses were performed to determine whether an inadequate platelet response to prescribed aspirin was related to a higher risk of recurrence of future vascular events.^{3,4} The odds ratio for a recurrent vascular event in patients with an inadequate aspirin response compared to aspirin-sensitive patients was 3.8 in both studies. The primary reports used as the basis for the meta-analysis in both studies were similar. The increased risk of a future vascular event was independent of the laboratory assay used to evaluate aspirin sensitivity. Of interest, the meta-analysis by Krasopoulos reported that patients with an inadequate aspirin response to prescribed aspirin did not benefit from an additional antiplatelet medication, clopidogrel.⁴

Mechanisms of Inadequate Platelet Response to Prescribed Aspirin

Noncompliance: The Most Common Cause of an Inadequate Aspirin Response

Noncompliance is the most important cause of an inadequate aspirin response to prescribed aspirin.^{8,15} Platelets from 17 of 192 patients with chronic coronary artery disease responded to AA stimulation in a light aggregometer, demonstrating that the platelets were not inhibited by the prescribed aspirin (Figure 2). These patients had been prescribed aspirin for at least 1 month. Repeat testing 2 hours after observed ingestion

Figure 2. Upper panel shows that 17 patients had an aggregometry response to arachidonic acid (AA) even though they had been instructed to take an aspirin daily for at least 1 month. If the patients had taken aspirin as prescribed, no aggregation response to AA would have been expected. Lower panel shows results obtained 2 hours after the patients were observed to take 325 mg of aspirin, all but 1 of the patients had the expected aspirin-induced no aggregation response to AA.

of 325 mg of aspirin showed inhibition of AA-stimulated light aggregometry in 16 of the 17 patients (Figure 2). These patients had an inadequate platelet response to aspirin because they did not take their prescribed aspirin. Of the 67 patients whose platelets had an aberrant response to aspirin, 62 were noncompliant. Among participants whose compliance was assured because they were observed to ingest aspirin, only 3% could be conservatively classified as aspirin resistant.¹⁵ Another study of 136 patients evaluated after coronary stenting with AA-stimulated light aggregometry identified 19 patients with an inadequate aspirin response.¹⁶ After observed aspirin ingestion 18 of the 19 responded to aspirin, showing that the initial nonresponse was also because of noncompliance. Both of these studies used repeat testing after observed aspirin ingestion to confirm that noncompliance was the main cause of the inadequate aspirin response (Table 1).

Using different experimental designs and different methods to detect aspirin's effect, 5 additional studies confirm that noncompliance with prescribed daily aspirin is the primary cause of an inadequate platelet response (Table 1). Verbal reinforcement of the importance of taking a daily aspirin coupled with repeated platelet function analyzer 100 (PFA-100; Siemens, USA) testing decreased the incidence of inadequate response to aspirin from 18% to 1.4%.¹⁷ This study confirms the importance of noncompliance and more importantly suggests that the strategy of verbal reminders coupled with repeat laboratory testing can markedly decrease the percentages of patients with inadequate platelet response to aspirin.¹⁷ Tandry studied patients prior to percutaneous coronary intervention. Of the 223 coronary arterial disease patients prescribed a daily aspirin, 7 demonstrated poor platelet inhibition with AA light transmission aggregometry and lack of platelet inhibition in the platelet phase of thromboelastography. After in-hospital treatment with 325 mg of aspirin, these 7 patients responded to aspirin as evidenced by inhibited AA light transmission aggregometry and thromboelastographic response.¹⁸ Initial evaluation of 73 post-MI patients prescribed a daily aspirin showed 21 (29%) failed to demonstrate decreased thromboxane B₂ (TXB₂) production. However, when questioned 12 of the 21 admitted to noncompliance.¹⁹ Frelinger studied 680 cardiac catheterization patients. Only 12 had partial reduction of serum TXB₂. Ex vivo addition of aspirin produced full reduction of serum TXB₂ and AA-stimulated light aggregation, suggesting that the initial inadequate aspirin response in these 12 patients was secondary to noncompliance.²⁰ Komiya used verbal confirmation to show that 10% of 157 outpatients with cerebral vascular disease had inadequate platelet inhibition to prescribed aspirin because of noncompliance.²¹ These studies demonstrate that when patients with inadequate platelet inhibition with aspirin are either retested under supervised conditions that assure compliance or are questioned specifically about taking their prescribed daily aspirin, the vast majority of patients have an inadequate response to aspirin because of noncompliance. Perhaps patients with proven noncompliance to prescribed aspirin should be labeled as "pseudo-aspirin resistant."

Other Clinically Relevant Causes of Inadequate Aspirin Response

Nonaspirin Nonsteroidal Anti-Inflammatory Agents Block Aspirin's Antiplatelet Effect

Taking a nonaspirin nonsteroidal anti-inflammatory agent (NANSAID) just before taking an aspirin blocks the antiplatelet effect of the aspirin.²² However, if the aspirin precedes the NANSAID, then aspirin-induced platelet inhibition is observed. Aspirin's antiplatelet effect is not blocked by rofecoxib, a cyclooxygenase 2 (COX-2) inhibitor, acetaminophen, or diclofenac.²³ While these ex vivo data are convincing, the question of whether concomitant administration of a

Table 1. Demonstration of Aspirin Noncompliance by Repeat Testing

N	% Noncompliant	ASA Effect	Methods for Repeat Testing for Compliance After	Reference
192	9.0	AA light aggregometry	Observed ASA ingestion	8
212	14.0	PFA-100	Strict reinforcement of compliance	17
203	3.4	Thromboelastography	Hospitalization	18
73	16.0	Thromboxane B ₂ : plasma	Admitted to noncompliance	19
87	20.0	Collagen light aggregometry	Admitted to noncompliance	21
678	2.0	AA light aggregometry	Ex vivo ASA	20
136	14	AA light aggregometry	Observed ASA ingestion	16

Abbreviations: AA = arachidonic acid; ASA = acetylsalicylic acid; PFA-100, platelet function analyzer.

NANSAID with aspirin increases the risk of future occlusive vascular events in patients is controversial. In the physician's health study, those participants taking aspirin who reported taking an NSAID on 60 or more days of the year had an increased relative risk of 2.86 for their first MI.²⁴ Patients with cardiovascular disease taking both aspirin and ibuprofen had an increased mortality with a hazard ratio of 1.93, when compared to patients taking aspirin alone.²⁵ An increased rate of MI was not observed for patients taking nonsteroidal anti-inflammatory drugs (NSAIDs) in a general population or for veteran's administration patients taking aspirin and NSAIDs.^{26,27} These data suggest that in patients with known vascular disease that the concomitant administration of aspirin and ibuprofen should be avoided. If the patient requires an NSAID, then aspirin should precede the NSAID. Diclofenac, acetaminophen, and rofecoxib do not block aspirin's antiplatelet effect and could be substituted for ibuprofen or another NSAID.

Increased Platelet Production

In theory, increased production of platelets will result in a greater proportion of nonaspirinated platelets decreasing the overall effect of aspirin-induced inhibition of platelet aggregation. Aspirin permanently inhibits COX-1 by acetylating the serine at position 539. Aspirin's acetylating activity is relatively short, lasting about 15 or 20 minutes, and is believed to take place in the portal vein before aspirin reaches the systemic circulation.^{28,29} Since the platelet life span is approximately 8 to 10 days, 24 hours after an oral aspirin about 90% of platelets are inhibited by aspirin acetylation and about 10% are new, nonaspirinated. Pharmacological investigations of platelet-related thromboxane A₂ (TXA₂) metabolites ex vivo and in urine suggest that biosynthesis of TX is maintained unless 95% or more inhibition of TX ex vivo is achieved.³⁰ Translation of these basic observations into clinical practice suggests that aspirin's clinical effect may be diminished in patients with increased platelet production rates. In addition, newly formed platelets have both COX-1 and COX-2 enzymes.³¹ Of interest, the proportion of COX-2-positive platelets increases from less than 10% observed in normals to a maximum of 60% in patients with increased platelet production. Cyclooxygenase 2 is much less sensitive to

aspirin inhibition than COX-1 and with the lower aspirin doses currently used COX-2 is less likely to be completely inhibited like COX-1.³² Since aspirin's inhibition of TXB₂ production in patients with increased platelet production is similar to that observed in normal participants, the clinical effect of COX-2 present in young platelets requires further investigation.³³

Increased platelet production probably is responsible for decreased platelet inhibition with aspirin that was observed after coronary bypass surgery. Five and 10 days after coronary bypass surgery diminished aspirin platelet inhibition was observed as evidenced by increased production of TXB₂ and increased AA-stimulated light aggregations. Immunoblots showed increased COX-2 5 days after surgery, possibly from newly formed platelets produced by surgical stimulation of increased platelet production.³⁴ In addition, patients with either carotid endarterectomy or leg angioplasty had decreased aspirin-induced platelet inhibition after surgery.³⁵ Perhaps the increased platelet consumption that is known to occur with surgery coupled with an increase in platelet production decreases the percentage of aspirinated platelets, resulting in a transitory state of inadequate platelet inhibition with aspirin.³⁶

From a clinician's perspective, an inadequate response to prescribed aspirin in a patient who is in the chronic phase of their obstructive arterial disease and has not had recent surgery and who is not also taking an NSAID is most likely caused by noncompliance. Reviews of those theoretical causes of inadequate response to prescribed aspirin that have not been demonstrated clinically can be found in the following references.^{5,37}

Assays to Detect the Effect of Aspirin on Platelet Function

The ideal assay to detect an inadequate aspirin response would be reliable, sensitive, specific, able to use whole blood, easy to use, yield an answer within a few minutes, be unaffected by platelet count between 100 000 and 400 000/ μ L, easily adaptable for use at the bedside or in the clinic and be inexpensive (Table 2). In addition, the group designated by the assay with an inadequate aspirin response should be shown with prospective studies to have an increased risk of future vascular events (Table 3).

Table 2. Ideal Assay for Detection of Inadequate Aspirin Response

1. Clearly differentiates aspirin-sensitive patients from aspirin-resistant
2. Reliable, sensitive, and specific
3. Technically easy
4. Provide an answer within a few minutes
5. Uses whole blood
6. Not affected by platelet concentrations between 100 000 and 400 000/ μ L
7. Allow patient testing at the bedside or as an outpatient
8. Be predictive of future vascular events
9. Inexpensive

Aspirin's main mechanism for delaying or preventing thrombosis is via inhibition of platelets. Cyclooxygenase 1 is an enzyme necessary for the metabolic conversion of AA to TXA₂ a potent platelet activator and vasoconstrictor which is rapidly converted to TXB₂ (Figure 3). Aspirin acetylates the serine at position 529 in the COX-1 enzyme, which irreversibly inactivates the enzyme.² Since platelets lack a nucleus, irreversible blockade of COX-1 by aspirin inhibits the platelet's function for the life of the platelet. Platelets in normal participants usually survive approximately 8 to 10 days.³⁸

Platelet inhibition produced by aspirin can be detected in vitro by assays that depend on activation of platelets or the measurement of TXA₂ metabolites.^{5,39}

Platelet Activation Techniques

Optical Aggregometry

Optical (light) aggregometry takes advantage of the phenomenon that a suspension of platelets in plasma appears cloudy (Figure 1). When the platelets are activated, they aggregate, allowing more light to be transmitted.⁴⁰ The optical aggregometer records the change in the transmission of light, recording the time it takes to begin aggregation, the slope of the aggregation curve, and the percentage of aggregation.⁴¹

Different agonists activate platelets by different mechanisms. Although platelet aggregometry is useful for investigative studies, it may not be cost-effective for routine evaluations of platelet function.

Arachidonic Acid-Stimulated Light Aggregometry (A Qualitative Assay)

Since aspirin selectively blocks the COX metabolic pathway, AA-stimulated platelet light aggregometry is the gold standard for measuring whether platelets are inhibited by aspirin. After a single 325 mg aspirin, platelet aggregation to AA stimulation is inhibited for 72 hours, while platelets not exposed to aspirin will aggregate normally (Figure 4).⁴² Arachidonic acid-induced light aggregometry is useful as a qualitative assay to determine whether platelets are or are not inhibited by aspirin.

Platelet Prostaglandin Agonist–Stimulated Light Aggregometry: A Semiquantitative Assay

Quantitative estimates of aspirin-induced inhibition with a light aggregometer require a different platelet agonist, one that is not exclusively dependent on aspirin's blockade of AA metabolism. Platelet prostaglandin agonist (PPA) is a proprietary agonist (Analytical Control Systems, Fishers, Indiana); the mechanism of activation is at least partially dependent on the AA pathway. Normal participants given 325 mg of aspirin demonstrate maximal inhibition of the slope of aggregation 2 hours after aspirin. The slope gradually increases over 72 hours toward baseline (Figure 4).⁴² This assay is useful in a semiquantitative sense to measure variations in the amount of aspirin-induced platelet inhibition. Aspirin inhibition of platelets decreases platelet function. Because the baseline excitation state of a platelet is dependent on stimulation from multiple receptors, it is highly variable.⁵ An accurate measure

Table 3. Mechanisms of an Inadequate Aspirin Response

1. Proven and clinically important
 - a. Noncompliance
 - b. Ibuprofen (NANSAIDs) interference with aspirin's COX-I-binding site
 - c. Clinical condition associated with increased platelet production
2. Theoretical mechanisms of aspirin resistance; not clinically proven causes of inadequate ASA response
 - a. Increased platelet stimulation with other, nonarachidonic acid–dependent mechanisms
 - i. ADP
 - ii. Collagen
 - iii. Epinephrine
 - b. Bypass of aspirin's blockage of platelet COX-I via COX-2 from neighboring cells
3. Unproven or controversial mechanisms
 - a. i. Undiscovered polymorphisms of COX-I that are resistant to aspirin
 - ii. Isoprostanes produced by free radical lipid peroxidation of AA
 - iii. Controversial association of platelet antigen PLA-1 or PLA-2 with aspirin resistance

Abbreviations: ADP = adenosine diphosphate; NANSAID = non-aspirin non-steroidal anti-inflammatory drug; PLA = platelet antigen; COX-I = cyclooxygenase I; AA = arachidonic acid.

Figure 3. Note that platelet prostaglandin agonist (PPA) demonstrates a gradual return of platelet aggregation to normal over 3 days. This characteristic of PPA-stimulated aggregation makes it useful for measuring gradations of aspirin-induced platelet inhibition. Arachidonic acid (AA) aggregation remains unresponsive for 3 days and returns to normal function between days 3 and 4. Arachidonic acid-stimulated platelet aggregations were used to show whether aspirin platelet inhibition was present or absent.

of the amount of aspirin-induced platelet inhibition must take into consideration the baseline platelet response off of aspirin.

Net Aspirin Response

The net aspirin response was defined as the difference in the slopes of the PPA-stimulated light aggregation curves obtained while the patients were off aspirin minus the slope of the aggregation curve obtained 2 hours after they were observed to ingest a 325 g aspirin tablet. Using PPA to measure net aspirin response, no difference was observed between normals and stable post-MI patients.¹⁵ The distribution of the net aspirin response for 192 participants observed to ingest aspirin fits the statistical definition for normal distribution which helps to substantiate the claim that PPA-stimulated light aggregometry quantitatively measures aspirin inhibition of platelets and demonstrates that participants with an inadequate response to prescribed aspirin do not constitute a separate population (Figure 5).

Figure 4. Aspirin blocks the metabolism of arachidonic acid (AA) to Prostaglandin A2 (PGA2) via covalent acetylation of cyclooxygenase I (COX-I), which irreversibly blocks the enzymatic activity (Adapted from Tischler, M. Internet lecture, Bio C801, University of Arizona).

Adenosine Diphosphate- and Collagen-Stimulated Light Aggregometry

Neither ADP- nor collagen-stimulated light aggregometry is a useful assay to measure aspirin-induced platelet inhibition. Both ADP and PPA were directly compared as agonists for light aggregometry (Figure 6).⁴² Participants were tested off aspirin and 2 hours after observed ingestion of 325 mg of aspirin. The slope of the PPA aggregation curve allowed 100% separation of the on and off aspirin platelet responses. No difference was observed between the on and off aspirin

Figure 5. When the single point with the largest aspirin response is removed as a statistical outlier, the net aspirin inhibitory response distribution curve is judged to be normally distributed.

Figure 6. Comparison of platelet prostaglandin agonist- (PPA) with adenosine diphosphate (ADP)-stimulated light aggregations 24 hours after volunteers ingested 325 mg of aspirin. The before and after aspirin slopes of PPA-stimulated aggregation are completely separated; no differences in the before and after aspirin slopes.

aggregation curves stimulated with ADP, demonstrating that ADP-induced platelet activation is not dependent on the AA pathway and that ADP light aggregometry should not be used to measure aspirin's antiplatelet effect. Collagen platelet activation is indirectly dependent on the AA metabolic pathway. However, variations in activation potency among batches of collagen make standardization difficult. Collagen-stimulated activation of platelets in a light aggregometer also cannot be recommended as an assay for measuring aspirin-induced platelet inhibition.

Prostaglandin Metabolites

Since aspirin blocks the metabolism of AA to TXA₂, measurements of metabolic products of TXA₂ in blood and urine have been used to assess aspirin's antiplatelet effect (Figure 3).⁵ However, blockade of TXA₂ production by aspirin is not specific for platelets. Thromboxane A₂ is quickly transformed to TXB₂ in blood and its metabolite 11-dehydro TXB₂ is excreted in urine and both of these metabolites of TXA₂ are used to measure platelet inhibition by aspirin.^{14,19,43} In addition to platelets, other cells that produce TXA₂ can also affect these measurements.

Point-of-Care Assays

Two devices, the PFA-100 and the Ultegra Rapid Platelet Function Analyzer (VerifyNow, Accumetrics, San Diego, California) are currently marketed as point-of-care assays designed for use in the clinic or at the bedside. Both devices use whole blood, are easy to use, and provide an answer quickly. The PFA-100 measures the time to occlusion, closure time of whole blood traveling through a flow cartridge coated

with collagen and epinephrine. In addition to being sensitive to aspirin-induced platelet inhibition, closure time can be decreased by increased levels of von Willebrand factor.⁴⁴ The VerifyNow assay uses whole blood and is dependent on change in optical density of fibrinogen-coated beads stimulated with AA. Correlations among different assays are poor.⁴⁵ In patients with stable coronary arterial disease treated with a minimum of 80 mg of aspirin per day the reported correlations between AA-stimulated light aggregometry and PFA-100 was -0.120 , between AA-stimulated light aggregometry and VerifyNow 0.151 and between PFA-100 and VerifyNow 0.189 . In comparison with the results obtained through investigations of stable coronary artery disease patients, normal volunteers taking 80 mg of aspirin per week demonstrated detectable platelet inhibition with 4 different assays including PFA-100 and VerifyNow.⁴⁶ In addition, the assay results with the 4 assays did not demonstrate increased aspirin inhibition when the dose was increased from 80 to 325 mg/d. Perhaps excellent compliance in the normal volunteers was responsible for the reliable decrease in platelet inhibition observed in all 4 assays. Compliance in a mixture of normal participants and patients with stable coronary artery disease was assured by complete suppression of serum TXB₂. Using the manufacturers suggested cutoff of ≥ 550 "aspirin resistance units" none of the participants were designated as having an inadequate aspirin response using the VerifyNow assay which compares to 12% classified with an inadequate aspirin response using AA-stimulated light aggregometry.⁴⁷ Agreement among 3 assays, AA-stimulated light aggregation, PFA-100, and VerifyNow, was evaluated in the acute phase of vascular insufficiency and repeated a year later when the patients were in the chronic phase. In both the acute and chronic phase of the disease agreement among the 3 assays was poor.⁴⁸

Concordance among assays used to identify patients with an inadequate aspirin response would be helpful. However, from a clinical perspective the larger question is, are the patients with an inadequate aspirin response using a particular assay at increased risk of a subsequent vascular event? The results with the PFA-100 are mixed. Some reports show that patients designated with an inadequate aspirin response using the PFA-100 are at an increased risk of a subsequent vascular event and some do not. In all, 97 patients with stable coronary arterial disease were evaluated 2.5 years after their initial event; those patients designated with an inadequate aspirin response by the PFA-100 assay did not have an increase in vascular events.⁴⁹ The PFA-100 designated 26 (20%) of 129 patients with ischemic cerebral vascular disease with an inadequate response to aspirin. After 56 months of follow-up, the rate of new ischemic events was the same in the group with an inadequate aspirin response as the aspirin-sensitive group.^{13,50} In comparison, 64 of the 496 patients with non-ST segment elevation acute coronary syndromes designated by the PFA-100 as having an inadequate aspirin response and followed for 1 year had an increased hazard ratio of 2.6 for cardiovascular death. Evaluation of stable coronary arterial disease

patients using the VerifyNow assay showed that 128 (27%) of 468 patients were designated as having an inadequate aspirin response. Patients with an inadequate aspirin response had an increased rate for a subsequent vascular event when compared to aspirin-sensitive patients with a hazard ratio of 3.1.¹³

Until reports with the PFA-100 consistently document an increased risk of occlusive vascular events in patients with an inadequate aspirin response, this technique cannot be recommended for routine clinical use. This is in accordance with the report from the International Society on Thrombosis and Scientific Standards.⁵¹ The data showing that VerifyNow designated inadequate aspirin-response patients are at increased risk of future vascular events is of interest, but the population studied was from Hong Kong, China. Before the VerifyNow results can be recommended for routine clinical use, the increased risk of future vascular events in inadequate aspirin responsive patients needs to be replicated using a Western population base.

An assay or device that is ideal does not exist. Light aggregation studies using AA as an agonist are helpful in determining whether platelet aggregation is inhibited by aspirin. The amount of aspirin-induced inhibition is estimated using PPA light aggregometry. However, light aggregation studies are expensive. Serum and urine determinations of prostaglandin A-2 metabolites help to confirm aspirin effect, but because other cells besides platelets can produce prostaglandin A-2, use of these assays to quantitatively measure aspirin inhibition of platelets are limited. The technical challenge is for the development of an assay to differentiate aspirin-compliant from -noncompliant patients.

Conclusions and Suggested Clinical Approach

1. Compliance is the main cause of an inadequate response to prescribed aspirin.
2. An inadequate response to prescribed aspirin is a common clinical problem with estimates of prevalence ranging from 4% to 83% with a consensus average of about 25%.
3. Two meta-analyses report that when compared to aspirin-sensitive patients, patients with an inadequate response to prescribed aspirin have a 3.8 times risk of a subsequent vascular event.
4. Prescribing clopidogrel to patients with an inadequate response to aspirin does not decrease their increased risk of a subsequent vascular event.

A clinician faced with a patient with an inadequate response to prescribed aspirin should focus on compliance. A single study checked the patients' response to aspirin using the PFA-100 at each office visit. Out of a cohort of 212 patients, 18% were initially classified with an inadequate aspirin response. One week after reinforcing the importance of compliance, the percentage of patients who still had an inadequate aspirin response was decreased to 10%. The patients were checked again 4 weeks

later and only 1.4% still had an inadequate response to their prescribed aspirin. This article suggests that a strategy of verbal re-enforcement coupled with a laboratory evaluation for aspirin effect can decrease the number of patients with an inadequate aspirin response by increasing compliance.¹⁷

The challenge for the future is the development and verification of a laboratory method to detect an inadequate response to prescribed aspirin that is quick, reliable, and inexpensive. Additional studies will be required to confirm that verbal reinforcement together with repeated laboratory evaluations can markedly decrease the prevalence of an inadequate response to prescribed aspirin. In addition, future studies are needed to determine whether transformation of a patient with an inadequate response to prescribed aspirin to an aspirin-sensitive patient via enforcement of compliance actually decreases the patient's risk of a future vascular event.

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