Aspirin Resistance: Current Concepts

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Aspirin is an effective antiplatelet agent with proven benefit in the prevention of atherothrombotic complications of cardiovascular disease. The antithrombotic effects of aspirin, however, are variable among individuals and this might explain, in part, why the absolute risk of recurrent vascular events in patients receiving aspirin therapy remains relatively high (8%–18% after 2 years). Although formal diagnostic criteria are lacking, aspirin resistance generally describes the failure of aspirin to produce an expected biological response or the failure of aspirin to prevent atherothrombotic events. Aspirin resistance has been reported to occur in 5% to 45% of the general population; therefore, its detection is potentially of clinical importance. The biological mechanisms, population prevalence, laboratory methods for detection, and clinical relevance of aspirin resistance are discussed in this review.
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Key words: Aspirin resistance • Coronary artery disease • Antiplatelet therapy

Aspirin is an effective antiplatelet agent with proven benefit in the prevention of atherothrombotic complications of cardiovascular disease. Clinical trials have demonstrated that aspirin is effective for both primary and secondary prevention of myocardial infarction, stroke, and cardiovascular death[1-3] and in the acute management of myocardial infarction, unstable angina, and embolic stroke.[3-5] A recent meta-analysis reported that among high-risk vascular patients, aspirin therapy was associated with a 34% reduction in nonfatal myocardial infarction, a 25% reduction in nonfatal stroke, and an 18% reduction

Table 1
Selected Studies Reporting the Prevalence of Aspirin Resistance

Investigators	N	Aspirin Dose (mg/d)	Methodology	Prevalence of Aspirin Resistance (%)
Grotemeyer et al[6]	180 (post-stroke)	100	Platelet reactivity: aggregation induced by blood collection	36
Helgason et al[30]	306 (post-stroke)	325	Optical platelet aggregometry using ADP, arachidonic acid, epinephrine, and collagen	25
Pappas et al[8]	31 (healthy adults)	325	Whole blood aggregation using arachidonic acid	N/A
Buchanan and Brister[7]	40 (CABG)	325	Bleeding time	43
Macchi et al[31]	72 (stable CAD)	160	PFA-100: defined ASA resistance as epinephrine closure time < 186 seconds	29.2
Andersen et al[32]	129 (stable CAD)	1. Aspirin (160) alone 2. Aspirin (75) plus Coumadin	PFA-100: defined ASA resistance as epinephrine closure time < 196 seconds	1. 35 2. 40
Wang et al[27]	422 (stable CAD)	325	RPFA: defined ASA resistance as ARU > 550	23.0
Gum et al[11]	325 (stable CAD)	325	1. Optical platelet aggregation: ADP and arachidonic acid 2. PFA-100 (collagen/ADP and collagen/epinephrine)	1. 5.5 2. 9.5
Chen et al[12]	151 (elective PCI)	80-325	RPFA: defined ASA resistance as ARU > 550	19.2

ADP, adenosine diphosphate; ARU, aspirin resistance units; ASA, aspirin; CABG, coronary artery bypass graft; CAD, coronary artery disease; PCI, percutaneous coronary intervention; PFA-100, platelet function analyzer; RPFA, rapid platelet function analyzer.

in all-cause mortality.[3] Despite this, the absolute risk of recurrent vascular events among patients taking aspirin remains relatively high, an estimated 8% to 18% after 2 years,[3] suggesting that the antiplatelet effects of aspirin may not be equivalent in all patients.

Measurements of platelet aggregation, platelet activation, and bleeding time have all confirmed variability in patients' antithrombotic responses to aspirin therapy.[6-9] Prospective studies have demonstrated that decreased responsiveness to aspirin therapy is associated with an increased risk of clinical events.[6,10-12] These observations and others have led to the concept of aspirin resistance. Although formal diagnostic criteria are lacking, aspirin resistance generally describes the failure of aspirin to produce an expected biological response (ie, platelet inhibition) or to prevent atherothrombotic events.

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The prevalence of aspirin resistance remains uncertain but the majority of previous studies have reported that it may affect anywhere from 5% to 45% of the population (Table 1). Given the prevalence of cardiovascular disease, aspirin resistance is potentially a significant clinical problem. Therefore, identifying aspirin nonresponders and achieving appropriate levels of platelet inhibition with alternate therapy potentially has great clinical importance. Recent clinical cardiovascular trials demonstrating the benefits of alternate antiplatelet agents, such as thienopyridine derivatives, highlight this fact.[4,13-15] In addition, it has been hypothesized that dual antiplatelet

therapy may have particular benefit among aspirin nonresponders.[16]

Mechanism of Action and Pharmacokinetics of Aspirin

Aspirin exhibits its primary antithrombotic effect by interfering with platelet aggregation. It does this by inhibiting the cyclooxygenase (COX) activity of prostaglandin (PG) H-synthase, which in turn blocks the metabolism of arachidonic acid to cyclic prostanoids such as thromboxane A2 (TXA2), prostacyclin, and other prostaglandins (Figure 1). Thromboxane A2 is synthesized and released by human platelets in response to a variety of stimuli (ie, collagen, adenosine diphosphate [ADP], thrombin, platelet activating factor) and acts to amplify the platelet aggregation response and to cause vasoconstriction (Figure 2). Inhibition of TXA2 formation is thought to be the primary mechanism for aspirin's antithrombotic effect and is achieved by the irreversible acetylation of the serine domain in the active site of COX. There are 2 COX isoforms but only the first (COX-1) is constitutively expressed in mature platelets. Because platelets have minimal capacity for protein synthesis, the inactivation of COX-1 by aspirin is irreversible for the life of the platelet (8-10 days). Patrono and colleagues[17] recently demonstrated that COX-2 is present in newly formed platelets (8%–10% of circulating platelets) and that PGE2 is the main product of platelet COX-2 activity. During periods of increased platelet turnover, however, the number of young platelets is large enough to produce detectable amounts of COX-2 derived TXA2.[18] COX-2 has been detected in a variety of cell types and tissue distributions. Its role in inflammatory disorders is widely recognized. The fact that aspirin is 170-fold more potent in inhibiting COX-1 than COX-2 explains in part why aspirin has relatively weak anti-inflammatory effects at low (81-325 mg/d) doses.[19]

Aspirin is rapidly absorbed from the gastrointestinal tract and peak

Figure 1. *Aspirin mediated inhibition of arachidonic acid metabolism. COX-1, cyclooxygenase isoform 1; HOX, hydroperoxidase; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; PG, prostaglandin; TXA2, thromboxane A2.*

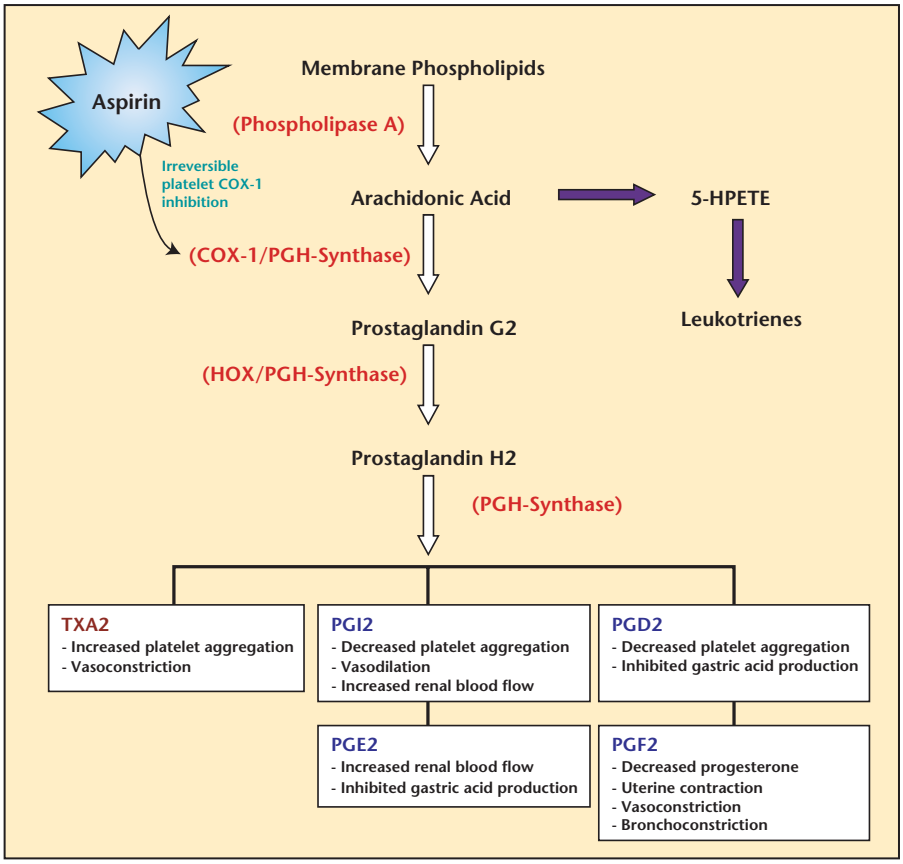

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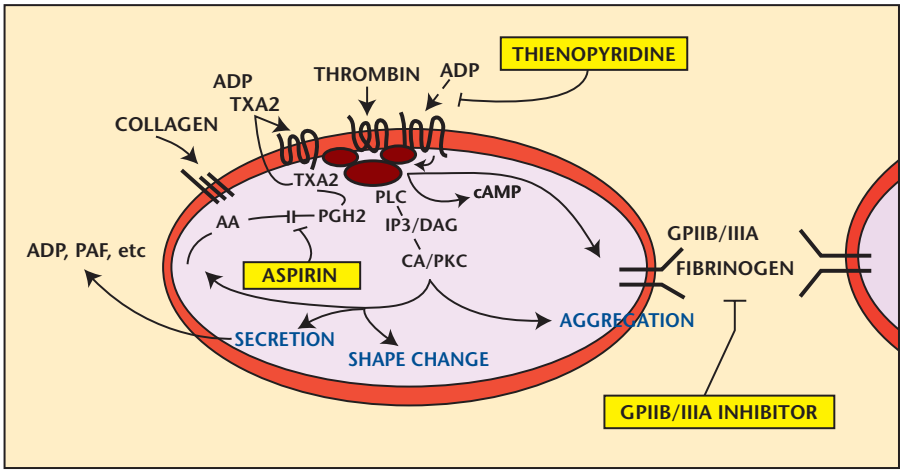
Figure 2. *Platelet activation. AA, arachidonic acid; ADP, adenosine diphosphate; CA, calcium; cAMP, cyclic-adenosine monophosphate; GP, glycoprotein; IP3/DAG, inositol–1,4,5–triphosphate/diacylglycerol; PAF, platelet activating factor; PG, prostaglandin; PKC, protein kinase C; PLC, phospholipase C; TXA2, thromboxane A2.*



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plasma concentrations are achieved in 30 to 40 minutes. Significant platelet inhibition is noted within 60 minutes of ingestion and a single dose of 100 mg of aspirin can completely block TXA2 production for the life of the platelet in most individuals.[20,21] The plasma half-life of aspirin is only 20 minutes but the irreversible nature of aspirin's effect on platelet function makes once-daily dosing sufficient to maintain its antithrombotic benefit. Although therapeutic benefits have been demonstrated for a variety of doses (30 to 1500 mg/d), higher dose regimens (500-1500 mg/d) are not associated with significant added benefit and have been associated with increased risk of adverse effects.[2,3] A recently published retrospective analysis of patients with acute coronary syndromes enrolled in the Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) IIb and Platelet IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy (PURSUIT) trials failed to demonstrate a significant difference in 6-month outcomes among patients taking low- (< 150 mg/d) and intermediate- (> 150 mg/d) dose aspirin.[22]

Aspirin might also influence hemostasis and cardiovascular disease by mechanisms independent of prostaglandin production. Although less clearly defined, the non-prostaglandin mediated effects of aspirin on hemostasis are thought to be dose-dependent and unrelated to COX-1 activity. These effects include vitamin K antagonism, decreased platelet production of thrombin, and acetylation of 1 or more clotting factors.[19] Aspirin might also impair platelet function by inhibiting neutrophil-mediated platelet activation.[19] In addition to its direct platelet effects, aspirin might alter the pathogenesis of cardiovascular disease by protecting LDL from oxidative modification, thereby improving endothelial dysfunction in atherosclerotic patients, and by attenuating the inflammatory response by acting as an antioxidant.[23]

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Measurement of Platelet Function

Traditionally, platelet aggregation has been measured in platelet-rich plasma using an optical aggregometer. The aggregation response is stimulated by the addition of a platelet agonist (ie, epinephrine, ADP, or collagen) and graded on a 0% to 100% scale, according to the degree of light transmission. As platelets bind via fibrinogen, light transmission increases. Although this technique has been used extensively, it is labor-intensive and requires technical expertise, and the results may vary with changes in platelet count and agonist used.[24] In contrast, whole blood aggregometry eliminates the need to prepare platelet-rich plasma and measures the platelet aggregation response using electrical impedance rather than optical density. The results of this technique, however, have not correlated well with optical aggregometry.[24]

Other means of analyzing platelet function include the platelet function analyzer (PFA)-100 (Dade-Behring, Deerfield, IL) and the Rapid Platelet Function Assay (RPFA) (Accumetrics, San Diego, CA).[25] Both of these methods are point-of-care tests intended for widespread clinical use. The PFA-100 system simulates hemostasis by allowing whole blood to flow through a cartridge containing an aperture coated with collagen and epinephrine or ADP. The time required for platelet plug formation, aperture closure, and cessation of blood flow is used as a measure of platelet activation. The PFA-100 system demonstrates reasonable correlation with optical aggregometry and has been used to measure platelet response to aspirin therapy.[9,26] The RPFA system is a turbidimetric-based optical detection system that measures platelet-induced aggregation in citrated whole blood. Cationic propyl gallate is used to induce platelet activation and platelet agglutination with fibrinogen-coated microparticles. Adhesion occurs in proportion to the number of available platelet receptors, is measured as an increase in light transmission, and is reported in aspirin response units (ARUs). Aspirin nonresponsiveness is defined by an ARU value greater than 550. The assay is affected by concomitant glycoprotein IIb/IIIa inhibitor, clopidogrel, dipyridamole, streptokinase, and non-steroidal anti-inflammatory drug (NSAID) therapy. Results from the RPFA system correlate well with those from the PFA-100 system as well as traditional optical aggregometry using platelet-rich plasma.[26] Recent studies have used the RPFA system to correlate aspirin nonresponsiveness with increased risk of cardiovascular events.[12,27]

Platelet activation can also be measured by the release of arachidonic acid metabolites. Urinary levels of

11-dehydro TXB2, a stable metabolite of TXA2, have been used to study the extent of aspirin mediated inhibition of thromboxane generation.[10] However, it is not known whether the persistent elevation in urinary TXB2 levels is explained simply by uninhibited platelet COX-1 activity or perhaps also by COX-1-independent sources of TXA2 generation. Serum markers, such as soluble CD40 ligand and P-selectin, have also shown promise as measurements of platelet activation.[28] To date, however, little is known about these markers and their correlation with platelet aggregometry, especially in the context of aspirin therapy.

Aspirin Resistance

The potential for therapeutic resistance originated from the observation that the immediate biological effects of aspirin are not uniform among all patients. Mehta and coworkers[29] reported that 30% of patients with coronary artery disease had minimal inhibition of platelet aggregation with a single 650 mg dose of aspirin. Similar variability in aspirin-mediated platelet inhibition was noted among normal subjects, post-stroke patients, patients with stable coronary artery disease, and patients presenting for coronary artery bypass surgery.[6-8,30-33] More recently, Grundmann and colleagues[33] reported that, in patients with symptomatic transient ischemic attack or stroke, the incidence of aspirin resistance was significantly higher (34%) as compared to a panel of asymptomatic patients with known cerebrovascular disease (0%). Despite the apparent consistency of these observations, the exact prevalence of aspirin resistance remains uncertain. The absence of standardized diagnostic criteria or a single validated method of identifying affected individuals has led to a wide range of population estimates (Table 1).

A number of studies have explored the relationship between platelet reactivity, aspirin therapy, and risk of future vascular events. Grotemeyer[34] reported a 30% incidence of aspirin resistance among post-stroke patients (as defined by a platelet reactivity index greater than 1.25) after the ingestion of 500 mg aspirin. At 2-year follow-up, the aspirin nonresponders had a 10-fold increase in the risk of recurrent vascular events as compared to aspirin-sensitive patients.[6] Similarly, aspirin resistance was found in 40% of patients with intermittent claudication who presented for a peripheral vascular angioplasty procedure. After 18 months of follow-up, aspirin resistance was associated with an 87% increase in the risk of arterial reocclusion.[35] In a nested case-control study among aspirin-treated patients within the Heart Outcome Prevention Evaluation (HOPE) trial, investigators found that the risk of myocardial infarction, stroke, or cardiovascular death increased with each increasing quartile of urinary 11-dehydro TXB2. Those in the upper quartile had a 2-fold increase in risk of myocardial infarction and a 3.5-fold increase in risk of cardiovascular death when compared to those in the lowest quartile.[10]

More recently, Gum and coworkers[11] reported a 5% incidence of aspirin resistance, as defined by optical platelet aggregation, among 326 patients with stable cardiovascular disease. With a mean follow-up of 2.1 years, they found that aspirin resistance was associated with a significant increase (hazard ratio 3.12, 95% confidence interval 1.1–8.9) in the risk of myocardial infarction, stroke, or death. Chen and colleagues[12] reported a 19.2% incidence of aspirin resistance, as defined by the Ultegra RPFA, among 151 patients with coronary artery disease who presented for a non-urgent percutaneous coronary intervention procedure. Despite adequate pretreatment with clopidogrel and procedural anticoagulation with heparin, aspirin resistance was associated with a 2.9-fold increased risk of CK-MB elevation, when compared to aspirin-sensitive patients.

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Observations from clinical trials involving patients with coronary artery disease who experience atherothrombotic events while on aspirin therapy also support the concept of aspirin resistance. Investigators from the PURSUIT trial reported that among 9461 patients presenting with acute coronary syndromes, those previously taking aspirin were 20% more likely to suffer a recurrent event in the following 6 months as compared to patients who were previously aspirin-naïve.[36] Similarly, a post-hoc analysis of the Platelet Receptor Inhibition in Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms (PRISM-PLUS) trial and a combined analysis of the Efficacy and Safety of Subcutaneous Enoxaparin in Non-Q-wave Coronary Events (ESSENCE) and the Thrombolysis in Myocardial Infarction 11B (TIMI 11B) trials reported that prior

aspirin use was an independent predictor of increased cardiovascular risk among patients with acute coronary syndromes.[37]

Potential Mechanisms of Aspirin Resistance

Although the mechanism for aspirin resistance remains uncertain, it is likely due to a combination of clinical, biological, and genetic properties affecting platelet function (Table 2). From the clinical perspective, behavioral habits (ie, tobacco use) as well as issues of copharmacy (eg, NSAIDs) may help contribute to individual differences in aspirin responsiveness. Tobacco use increases platelet activation and accentuates platelet thrombus formation despite aspirin-mediated suppression of platelet COX-1 activity and TXA2 synthesis.[38] Although controversial, several studies have demonstrated that the coadministration of aspirin with NSAIDs may attenuate the long-term antithrombotic benefits of aspirin.[39,40] Nonselective NSAIDs have a strong binding affinity for a specific region of platelet COX-1 and may prevent aspirin mediated acetylation and enzyme inhibition. Current data are not, however, consistent or definitive in proving the clinical relevance of this potential interaction.

The duration of aspirin therapy may also contribute to aspirin responsiveness. A recent study investigated ADP and collagen-induced platelet aggregation before and after 2, 6, 12, and 24 months of aspirin treatment (100 or 330 mg/d) in 150 subjects. Despite an adequate platelet-inhibitory response at 2 months, long-term treatment with aspirin was associated with progressive reduction in sensitivity to its effects.[41]

Platelets are activated by multiple receptors and pathways and this redundancy may contribute to the problem of aspirin resistance. Thus, pathways involving non-TXA2-dependent activators such as thrombin, ADP, epinephrine, and collagen can bypass the aspirin-mediated inhibitory effect leading to platelet activation and thrombosis. Aspirin may not adequately inhibit catecholamine-induced platelet aggregation. Among patients with a previous myocardial infarction, it was demonstrated that although aspirin achieved adequate antiplatelet effects at rest, it failed to inhibit exercise-induced increases in platelet aggregation.[42] Among normal subjects, aspirin pretreatment was shown to attenuate basal platelet aggregability but not the effect of norepinephrine infusion on platelet aggregation.[43] Both observations suggest that the antiplatelet effects of aspirin may be overcome during times of increased sympathetic nervous system activity, including periods of exercise or mental stress. Finally, there is evidence to suggest that aspirin nonresponders may have increased platelet sensitivity to collagen.[44]

Alternate pathways for synthesis of TXA2 and prostaglandin-like compounds (isoprostanes) have been identified and may contribute to the syndrome of aspirin resistance. The interaction of platelets with other

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Table 2
Potential Mechanisms of Aspirin Resistance

Clinical Variables:

1) Cigarette smoke enhances platelet function
2) Non-steroidal anti-inflammatory drugs inhibit aspirin-mediated COX-1 acetylation and may attenuate the long-term antithrombotic benefits of aspirin
3) Subtherapeutic aspirin levels: Antiplatelet effects may be dose dependent

Biological Factors:

1) Alternate pathways of platelet activation:
   a) Increased collagen sensitivity in aspirin nonresponders may lead to increased platelet adhesion
   b) Failure to inhibit catecholamine-mediated platelet activation (eg, exercise, mental stress, epinephrine)
2) Aspirin-insensitive TXA2 biosynthesis: Inducible COX-2 or regenerated COX-1 activity in macrophages and vascular endothelial cells may augment TXA2 production
3) Isoprostanes: Prostaglandin-like compounds can be produced from arachidonic acid and lipid peroxidation

Genetic Variables:

1) Mutations and/or polymorphisms of the COX-1 gene may prevent aspirin-mediated COX-1 acetylation
2) Glycoprotein IIb/IIIa receptor polymorphisms (PlA2)

COX-1, cyclooxygenase isoform 1; COX-2, cyclooxygenase isoform 2; TXA2, thromboxane A2.

cells such as erythrocytes may also affect aspirin-mediated inhibition as erythrocytes induce an increase in platelet TXB2 synthesis and release of serotonin, ß-thromboglobulin, and ADP. Previous investigation among patients with known vascular disease demonstrated that aspirin (200 to 300 mg daily) incompletely blocks platelet reactivity in up to two thirds of patients, in the presence of erythrocytes, despite adequate inhibition of platelet TXA2 synthesis.[45] Aspirin-insensitive TXA2 biosynthesis can occur as a result of COX-2 induction in non-platelet cells (monocytes/macrophages or endothelial cells) resulting from local inflammatory stimuli.[19,46] These cells can either release TXA2 or provide its precursor, PGH2, to the aspirin inhibited platelets. Additionally, COX-2 is present in newly formed platelets and may account for detectable levels of TXA2 synthesis during periods of increased platelet turnover.[18] Isoprostanes, resulting from lipid peroxidation, circulate at increased concentrations in patients with unstable angina, diabetes mellitus, and/or hyperlipidemia, and in cigarette smokers. In addition, acting as vasoconstrictors, isoprostanes may have a role in amplifying the response of platelets to other agonists.[17,47]

Lastly, it has been proposed that genetic differences in the COX-1 gene or the glycoprotein IIb/IIIa receptor complex may contribute to aspirin resistance. Polymorphisms of the IIIa subunit have been identified and specific alleles, PlA1/A2 and PlA2/A2, are associated with increased thrombin formation and a lower threshold for platelet activation, α-granule release, and fibrinogen binding. Carriers of the PlA2 polymorphism may be more resistant to the antithrombotic effects of aspirin.[48,49] Although unproven, it has been suggested that mutations and/or polymorphisms of the COX-1 gene may also help to explain the structural basis for aspirin resistance in some patients.[19]

Conclusions

Although the term aspirin resistance remains broadly defined, it appears to represent a valid and important biological phenomenon with significant clinical implications. There are, however, many unanswered questions. Principal among the uncertainties are: 1) the lack of a standardized definition and validated method of identifying aspirin resistance; 2) the unknown prevalence of aspirin resistance within the population; 3) the absence of a clearly defined biological mechanism for aspirin resistance; 4) the uncertain clinical relevance of aspirin resistance in cardiovascular risk prevention; and 5) the absence of a proven therapeutic intervention for affected individuals. While some of these questions will hopefully be addressed in the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization Management and Avoidance (CHARISMA) trial, an ongoing randomized clinical trial evaluating the combination of aspirin plus clopidogrel versus aspirin alone in both secondary prevention and high-risk primary prevention, further investigation is necessary before the concept of aspirin resistance can be readily applied to clinical practice and risk stratification. Until then, aspirin remains a proven and powerful therapy against the atherothrombotic complications of cardiovascular disease. ■

References

1. Steering Committee of the Physicians' Health Study Research Group. Final Report on the

Main Points

- Aspirin is an effective antiplatelet agent with proven benefit in the prevention of atherothrombotic complications of cardiovascular disease; however, the absolute risk of recurrent vascular events among patients taking aspirin remains relatively high, suggesting that the antiplatelet effects of aspirin may not be equivalent in all patients.
- Although formal diagnostic criteria are lacking, aspirin resistance generally describes the failure of aspirin to produce an expected biological response (ie, platelet inhibition) or to prevent atherothrombotic events.
- Aspirin resistance has been estimated to exist in anywhere from 5% to 45% of the population, representing a phenomenon of possible clinical significance.
- Traditionally, platelet aggregation has been measured in platelet-rich plasma using an optical aggregometer. Other effective means of analyzing platelet function include the platelet function analyzer (PFA)-100 and the rapid platelet function assay (RPFA).
- Unresolved issues regarding aspirin resistance include the absence of a clearly defined biological mechanism for the phenomenon, the uncertain clinical relevance of aspirin resistance in cardiovascular risk prevention, and the absence of a proven therapeutic intervention for affected individuals.

aspirin component of the ongoing Physicians' Health Study. *N Engl J Med*. 1989;321:129-135.
2. Antiplatelet Trialists' Collaboration. Collaborative overview of randomized trials of antiplatelet therapy. I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ*. 1994;308:81-106.
3. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ*. 2002;324:71-86.
4. Yusuf S, Zhao F, Mehta SR, et al. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST segment elevation. *N Engl J Med*. 2001; 345:494-502.
5. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. *Lancet*. 1988;13:349-360.
6. Grotemeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of aspirin responder and aspirin non responder. A pilot-study including 180 post-stroke patients. *Thromb Res*. 1993;71:397-403.
7. Buchanan MR, Brister SJ. Individual variation in the effects of ASA on platelet function: implications for the use of ASA clinically. *Can J Cardiol*. 1995;11:221-227.
8. Pappas JM, Wistengard JC, Bull BS. Population variability in the effect of aspirin on platelet function. Implications for clinical trials and therapy. *Arch Pathol Lab Med*. 1994;118:801-804.
9. Gum PA, Kottke-Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. *Am J Cardiol*. 2001;88:230-235.
10. Eikelboom JW, Hirsh J, Weitz JI, et al. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation*. 2002;105:1650-1655.
11. Gum PA, Kottke-Marchant K, Welsh PA, et al. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol*. 2003;41:961-965.
12. Chen W-H, Lee P-Y, Ng W, et al. Aspirin resistance is associated with a high incidence of myonecrosis after non-urgent percutaneous coronary intervention despite clopidogrel pretreatment. *J Am Coll Cardiol*. 2004;43:1122-1126.
13. CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischemic events (CAPRIE). *Lancet*. 1996;348:1329-1339.
14. Mehta SR, Yusuf S, Peters RJG, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet*. 2001;358:527-533.
15. Steinhubl SR, Berger PB, Mann III JT, et al for the CREDO Investigators. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: A randomized controlled trial. *JAMA*. 2002;288:2411-2420.
16. Jneid H, Bhatt DL, Corti R, et al. Aspirin and clopidogrel in acute coronary syndromes: Therapeutic insights from the CURE study. *Arch Intern Med*. 2003:163;1145-1153.
17. Cipollone F, Rocca B, Patrono C. Cyclooxygenase-2 expression and inhibition in atherothrombosis. *Arterioscler Thromb Vasc Biol*. 2004;24:246-255.
18. Rocca B Secchiero P, Ciabattoni G, et al. Cyclooxygenase-2 expression is induced during human megakaryopoiesis and characterizes newly formed platelets. *Proc Natl Acad Sci*. 2002;99:7634-7639.
19. Patrono C, Coller B, Dalen JE, et al. Platelet-active drugs: the relationships among dose, effectiveness, and side effects. *Chest*. 2001; 119:39S-63S.
20. Patriginani P, Filabozzi P, Patrono C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. *J Clin Invest*. 1982;69: 1366-1372.
21. Weksler BB, Pett SB, Alonso D, et al. Differential inhibition by aspirin of vascular and platelet prostaglandin synthesis in atherosclerotic patients. *N Engl J Med*. 1983; 308(14):800-805.
22. Quinn MJ, Aronow HD, Califf RM, et al. Aspirin dose and six-month outcome after an acute coronary syndrome. *J Am Coll Cardiol*. 2004;43:972-978.
23. Awtry EH, Loscalzo J. Aspirin. *Circulation*. 2000;101:1206-1218.
24. Nicholson NS, Panzer-Knodle SG, Haas NF, et al. Assessment of platelet function assays. *Am Heart J*. 1998;135:S170-178.
25. Homoncik M, Jilma B, Hergovich N, et al. Monitoring of aspirin (ASA) pharmacodynamics with the platelet function analyzer PFA-100. *Thromb Haemost*. 2000:82;316-321.
26. Malinin AI, Atar D, Callahan KP, et al. Effect of a single dose aspirin on platelets in humans with multiple risk factors for coronary artery disease. *Eur J Pharmacol*. 2003;462:139-143.
27. Wang JC, Aucoin-Barry D, Manuelian D, et al. Incidence of aspirin nonresponsiveness using the Ultegra Rapid Platelet Function Assay-ASA. *Amer J Cardiol*. 2003;92:1492-1494.
28. Freedman JE. CD40-CD40L and platelet function: beyond hemostasis. *Circ Res*. 2003; 92:944-946.
29. Mehta J, Mehta P, Burger C, et al. Platelet aggregation studies in coronary artery disease. Part 4. Effect of aspirin. *Atherosclerosis*. 1978; 31(2):169-175.
30. Helgason CM, Bolin KM, Hoff JA, et al. Development of aspirin resistance in persons with previous ischemic stroke. *Stroke*. 1994; 25: 2331-2336.
31. Macchi L, Christiaens L, Brabant S, et al. Resistance to aspirin in vitro is associated with increased platelet sensitivity to adenosine diphosphate. *Thromb Res*. 2002;107:45-49.
32. Andersen K, Hurlen M, Arnesen H, et al. Aspirin non-responsiveness as measured by PFA-100 in patients with coronary artery disease. *Thromb Res*. 2002;108:37-42.
33. Grundmann K, Jaschonek K, Kleine B, et al. Aspirin non-responder status in patients with recurrent cerebral ischemic attacks. *J Neurol*. 2003;250:63-66.
34. Grotemeyer KH. Effects of acetylsalicylic acid in stroke patients. Evidence of nonresponders in a subpopulation of treated patients. *Thromb Res*. 1991; 63(6):587-593.
35. Mueller MR, Salat A, Stangl P, et al. Variable platelet response to low-dose ASA and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. *Thromb Haemost*. 1997;78:1003-1007.
36. Alexander JH, Harrington RA, Tuttle RH, et al. Prior aspirin use predicts worse outcomes in patients with non-ST-elevation acute coronary syndromes. PURSUIT Investigators. Platelet IIb/IIIa in Unstable angina: Receptor Suppression Using Integrilin Therapy. *Am J Cardiol*. 1999;83(8):1147-1151.
37. Antman EM, Cohen M, Bernink PJ, et al. The TIMI risk score for unstable angina/non-ST elevation MI: a method for prognostication and therapeutic decision making. *JAMA*. 2000; 284:835-842.
38. Hung J, Lam JYT, Lacoste L, et al. Cigarette smoking acutely increases platelet thrombus formation in patients with coronary artery disease taking aspirin. *Circulation*. 1995; 92:2432-2436.
39. Catella-Lawson F, Reilly MP, Kapoor SC, et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *N Engl J Med*. 2001;345:1809-1817.
40. Kurth T, Glynn RJ, Walker AM, et al. Inhibition of clinical benefits of aspirin on first myocardial infarction by nonsteroidal anti-inflammatory drugs. *Circulation*. 2003; 108:1191-1195.
41. Pulcinelli FM, Pignatelli P, Celestini A, et al. Inhibition of platelet aggregation by aspirin progressively decreases in long-term treated patients. *J Am Coll Cardiol*. 2004;43:979-984.
42. Hurlen M, Seljeflot I, Arnesen H. Increased platelet aggregability during exercise in patients with previous myocardial infarction. Lack of inhibition by aspirin. *Thromb Res*. 2000;99(5):487-494.
43. Larsson PT, Wallen NH, Hjemdahl P. Norepinephrine-induced human platelet activation in vivo is only partly counteracted by aspirin. *Circulation*. 1994;89(5):1951-1957.
44. Kawasaki T, Ozeki Y, Igawa T, et al. Increased platelet sensitivity to collagen in individuals resistant to low-dose aspirin. *Stroke*. 2000; 31:591-595.
45. Valles J, Santos MT, Aznar J, et al. Erythrocyte promotion of platelet reactivity decreases the effectiveness of aspirin as an antithrombotic therapeutic modality: the effect of low-dose aspirin is less than optimal in patients with vascular disease due to prothrombotic effects of erythrocytes on platelet reactivity. *Circulation*. 1998;97:350-355.
46. Maclouf J, Folco G, Patrono C. Eicosanoids and iso-eicosanoids: constitutive, inducible and transcellular biosynthesis in vascular disease. *Thromb Haemost*. 1998;79:691-705.
47. Morrow JD, Hill KE, Burk RF, et al. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci USA*. 1990;87:9383-9387.
48. Undas A, Brummel K, Musial J, et al. Pl(A2) polymorphism of beta(3) integrins is associated with enhanced thrombin generation and impaired antithrombotic action of aspirin at the site of microvascular injury. *Circulation*. 2001;104:2666-2672.
49. Michelson AD, Furman MI, Goldschmidt-Clermont P, et al. Platelet GP IIIa Pl(A) polymorphisms display different sensitivities to agonists. *Circulation*. 2000;101:1013-1018.