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Aspirin Resistance and Atherothrombotic Disease

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Acute coronary syndromes and other manifestations of atherothrombotic disease are primarily caused by atherosclerotic plaque rupture or fissuring and subsequent occlusive or subocclusive thrombus formation. Platelets play a critical role in the pathophysiology of atherothrombotic disease, and aspirin is the most commonly used antiplatelet agent. Clinical trials have demonstrated the efficacy of aspirin in both primary and secondary prevention of myocardial infarction, stroke, and cardiovascular death. Despite its proven benefit, the absolute risk of recurrent vascular events among patients taking aspirin remains relatively high, an estimated 8% to 18% after two years. Therapeutic resistance to aspirin might explain a portion of this risk. Although formal diagnostic criteria and a validated method of measurement are lacking, aspirin resistance may affect between 5% and 45% of the population. Given the prevalence of cardiovascular disease, the potential impact of aspirin resistance is large. Currently, however, there are many unanswered questions regarding the biological mechanism, diagnosis, population prevalence, clinical relevance, and optimal therapeutic intervention for aspirin resistance. (J Am Coll Cardiol 2005;46:986–93) © 2005 by the American College of Cardiology Foundation

The spectrum of acute coronary syndromes including unstable angina, non–ST-segment elevation myocardial infarction (MI), ST-segment elevation MI, and sudden death account for more than two million hospitalizations and 30% of all deaths in the U.S. each year. The majority of acute coronary syndromes are caused by atherosclerotic plaque rupture or fissuring and subsequent occlusive or subocclusive thrombus formation. Plaque rupture exposes the contents of the lipid core and promotes platelet adhesion and activation of the extrinsic coagulation cascade. Activated platelets then release a variety of vasoactive substances, including thromboxane (TX)A_2 and adenosine diphosphate (ADP), that promote platelet aggregation and primary hemostasis. Secondary hemostasis occurs as a result of thrombin-mediated conversion of fibrinogen to fibrin and subsequent stabilization of the platelet aggregate.

In atherothrombosis, the most commonly used inhibitor of platelet function is aspirin. The potential antithrombotic effects of aspirin were first reported in the *Mississippi Valley Medical Journal* in 1953 (1). Since that time, numerous investigations have contributed to our understanding of aspirin's antiplatelet effects and its potential role in the treatment of atherothrombotic disease. Clinical trials have subsequently demonstrated that aspirin is effective for both primary and secondary prevention of MI, stroke, and cardiovascular death (2,3) and in the acute management of MI, unstable angina, and embolic stroke (4–6). A recent meta-analysis reported that, among high-risk vascular patients, aspirin therapy was associated with a 34% reduction in nonfatal MI, a 25% reduction in nonfatal stroke, and an 18% reduction in all-cause mortality (4). Atherothrombosis, however, is a complex physiological process, and the absolute risk of recurrent vascular events among patients taking aspirin remains relatively high, an estimated 8% to 18% after two years. This suggests that the antiplatelet effects of aspirin may not be equivalent in all patients and/or that multiple therapeutic agents may be necessary to effectively block platelet function (Fig. 1).

Measurements of platelet aggregation, platelet activation, and bleeding time have all confirmed variability in patients' antithrombotic responses to aspirin therapy (7–10). Prospective clinical studies have demonstrated that decreased responsiveness to aspirin therapy is associated with an increased risk of atherothrombotic events (7,11–13). Recent cardiovascular trials demonstrating the benefits of alternate antiplatelet agents such as the thienopyridine derivatives, used independently or in combination with aspirin, highlight the clinical importance of achieving appropriate levels of platelet inhibition in preventing atherothrombosis (5,14–16). These observations and others have contributed 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 (i.e., platelet inhibition) or the failure of aspirin to prevent atherothrombotic events.

Given the prevalence of cardiovascular disease, the potential impact of aspirin resistance is large. Although the prevalence of aspirin resistance remains uncertain, previous studies have reported that it may affect between 5% and 45% of the population (Table 1). Therefore, identifying aspirin nonresponders and achieving appropriate levels of platelet inhibition with alternate therapy might have significant clinical impact. It has been hypothesized, for example, that dual antiplatelet therapy may have particular utility among

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Abbreviations and Acronyms

ADP	= adenosine diphosphate
CAD	= coronary artery disease
COX	= cyclooxygenase
CRP	= C-reactive protein
MI	= myocardial infarction
NSAIDs	= non-steroidal anti-inflammatory drugs
PFA	= platelet function analyzer
PG	= prostaglandin
PGH_2	= prostaglandin H_2
PGI_2	= endothelium-derived prostacyclin
PURSUIT	= Platelet IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy trial
RPFA	= rapid platelet function assay
TX	= thromboxane

aspirin-resistant patients (17). Currently, there are many unanswered questions regarding the biological mechanism, diagnosis, clinical relevance, and treatment of aspirin resistance.

ASPIRIN

Mechanism of action. The antiplatelet effects of aspirin are well-described and have been reviewed elsewhere (18,19). Aspirin achieves its primary antithrombotic effect by interfering with platelet aggregation, and it does this by inactivation of cyclooxygenase (COX), a key enzyme in platelet arachidonate metabolism. More specifically, aspirin inhibits the COX activity of prostaglandin (PG) H-synthase, which in turn blocks the metabolism of arachidonic acid to prostaglandin H_2 (PGH_2), the precursor of TXA_2 and other cyclic prostanoids (prostacyclin and other PGs). In human platelets, TXA_2 is synthesized and released in response to a variety of stimuli (i.e., collagen, ADP, thrombin, platelet activating factor) and acts to amplify the activation signal, promote irreversible platelet aggregation, and cause vasoconstriction (Fig. 1). Cyclooxygenase activity is inhibited by aspirin via the acetylation of a single serine residue at position 529 (Ser^{529}) within platelet PGH-synthase. There are two 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 to 10 days). The second COX isoform (COX-2) is inducible in newly formed platelets (8% to 10% of circulating platelets), and prostaglandin E_2 is the main product of platelet COX-2 activity (20). The concentration of newly formed platelets is large enough during periods of increased platelet turnover to produce detectable amounts of COX-2–derived TXA_2 (21); however, the clinical relevance of these observations is unknown. Cyclooxygenase-2 has been detected in a variety of cell types, tissue distributions, and its role in inflammatory disorders is widely recognized. The relatively weak anti-inflammatory effect of aspirin at low doses (81 to 325 mg/day) is in part explained by the fact that aspirin has 170-fold more potent inhibition of COX-1 than COX-2 (18).

Platelet adhesion and aggregation are inhibited by a

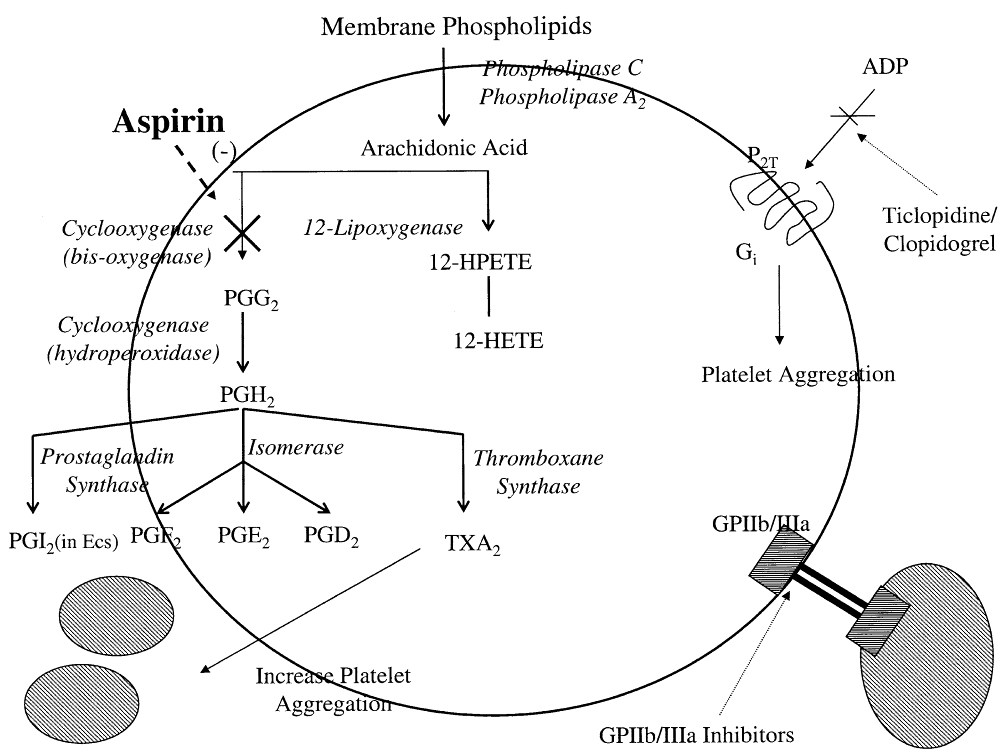

Figure 1. Platelet function and mechanisms of antiplatelet therapy. ADP = adenosine diphosphate; Ecs = endothelial cells; G_i = inhibitory G protein; GP = glycoprotein; PG = prostaglandin; P2 = type 2 platelet purinergic receptor; TX = thromboxane; HETE = hydroxyeicosatetraenoic acid; HPETE = hydroperoxyeicosatetraenoic acid.

Table 1. Prevalence of Aspirin Resistance

Population	Study Size	Aspirin (mg/day)	Measurement of Platelet Function	Aspirin Resistance
Healthy subjects				
Marshall et al. (38)	n = 12	750 three times/day	PFA-100	8.3%
Pappas et al. (9)	n = 31	325	Whole blood aggregometry using ADP, arachidonic acid, epinephrine, and collagen	N/A
Cerebrovascular disease				
Grotemeyer et al. (7)	n = 180	500	Platelet reactivity: aggregation induced by blood collection	36%
Helgason et al. (40)	n = 306	325	Optical platelet aggregometry using ADP, arachidonic acid, epinephrine, and collagen	25%
Grundmann et al. (41)	Symptomatic, n = 35 Asymptomatic, n = 18	100	PFA-100	Symptomatic, 34% Asymptomatic, 0%
Peripheral vascular disease				
Mueller et al. (45)	n = 100	325	Whole blood aggregation response to ADP and collagen agonists	60%
CAD				
Buchanan et al. (8)	CABG, n = 40	325	Bleeding time	43%
Macchi et al. (39)	Stable CAD, n = 72	160	PFA-100: defined ASA resistance as epinephrine closure time ≤186 s	29.2%
Andersen et al. (43)	Stable CAD, n = 129	1. Aspirin alone (160) 2. Aspirin (75) plus warfarin	PFA-100: defined ASA resistance as epinephrine closure time ≤196 s	Aspirin alone, 35% Aspirin plus warfarin, 40%
Wang et al. (35)	Stable CAD, n = 422	325	RPFA: defined ASA resistance ARU ≥550	23%
Gum et al. (12)	Stable CAD, n = 325	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. (13)	Elective PCI, n = 151	80–325	RPFA: defined ASA resistance ARU ≥550	19.2%

ADP = adenosine diphosphate; ARU = aspirin resistance units; ASA = aspirin; CABG = coronary artery bypass grafting; CAD = coronary artery disease; PCI = percutaneous coronary intervention; RPFA = rapid platelet function assay.

number of endogenous mechanisms including endothelium-derived prostacyclin (PGI_2), nitric oxide, and platelet cell molecule-1. Aspirin inhibits endothelium-derived PGI_2 production in a dose-dependent manner and may thereby antagonize its antiplatelet effects. Unlike platelets, however, endothelial cells rapidly recover COX activity and make this aspirin-mediated effect short-lived, dose-dependent, and perhaps less important when compared to the antiplatelet effect (17).

Aspirin may also influence hemostasis and cardiovascular disease by mechanisms independent of PG production. Although less clearly defined, the non–PG-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 one or more clotting factors (18). Aspirin might also impair platelet function by inhibiting neutrophil-mediated platelet activation (19). In addition to its direct platelet effects, aspirin could potentially alter the pathogenesis of cardiovascular disease by protecting low-density lipoprotein from oxidative modification, improving endothelial dysfunction in atherosclerotic patients, and by attenuating the inflammatory response by acting as an antioxidant (19).

The anti-inflammatory properties of aspirin are intriguing but not well-understood. A nested case-control study within the Physician Health study demonstrated that the cardioprotective effects of aspirin were primarily observed among men whose C-reactive protein (CRP) levels were within the highest quartile and that men with CRP levels in the lowest quartile had a lesser, nonsignificant benefit (22). Subsequent prospective studies have failed to demonstrate a consistent relationship between low- and moderate-dose aspirin therapy and reduction in CRP levels (23–25). However, pretreatment with aspirin has been shown to inhibit inflammation-mediated endothelial dysfunction, although the mechanism for this effect is unknown (26). Additionally, in healthy subjects, low-dose aspirin therapy has been shown to reduce platelet release of interleukin-7 and to reduce plasma levels of this cytokine (27). These findings suggest that part of aspirin's benefit might be mediated through a reduction in vascular inflammation.

Pharmacology. The pharmacokinetic properties of aspirin are marked by rapid absorption from the gastrointestinal tract and peak plasma concentrations within 30 to 40 min of ingestion. A significant platelet inhibitory effect is noted within 60 min of ingestion, and a single dose of 100 mg of aspirin can completely block TXA_2 production for the life of the platelet in most individuals (28,29). The plasma half-life of aspirin is approximately 20 min, but the irreversible nature of aspirin's inhibition of platelet COX-1 activity and duration of TXA_2 suppression means that the antithrombotic effects of aspirin are maintained with dosing intervals of 24 to 48 h (30).

Randomized trials have demonstrated that aspirin's therapeutic benefits are achieved from a variety of doses (30 to 1,500 mg/day), but the optimal daily dose has not been unequivocally determined (3,4). In general, higher dose regimens (500 to 1,500 mg/day) are not associated with significant added benefit, might actually attenuate the antithrombotic effect of aspirin, and have been associated with increased risk of adverse effects (18). 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 six-month outcomes among patients taking low-dose (<150 mg/day) and intermediate-dose (≥150 mg/day) aspirin (31).

Measurement of platelet function. Assessment of platelet function is complex. In response to activating stimuli, platelets may release a variety of chemokines, cytokines, and growth factors within preformed granules; synthesize prostanoids from arachidonic acid; or translate constitutively expressed messenger ribonucleic acid into proteins. In addition to playing a critical role in hemostasis, platelets likely participate in inflammatory pathways and the response to vascular injury. Despite the complexity of platelet function, laboratory methods for quantifying aspirin's antithrombotic effect have primarily focused on the measurement of platelet aggregation. Whether laboratory measurement of platelet aggregation fully encapsulates aspirin's biological effect, however, remains somewhat uncertain, and perhaps doubtful.

Platelet aggregation. Platelet aggregation is traditionally measured in platelet-rich plasma using an optical aggregometer. The aggregation response is stimulated by the addition of a platelet agonist (i.e., 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 used extensively, this technique is labor-intensive, requires technical expertise, and the results may vary with changes in platelet count and agonist used (32). Alternatively, 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 (32).

Point-of-care tests have been developed in an attempt to more easily measure platelet function and to monitor the effects of antiplatelet therapy. The platelet function analyzer (PFA)-100 system (Dade-Behring, Deerfield, Illinois) simulates hemostasis by flowing whole blood through a cartridge that contains an aperture coated with collagen and epinephrine or ADP (33). 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 (10,33,34). Another point-of-care test, the rapid platelet function assay (RPFA) (Accumetrics, San Diego, California), is a turbidimetric-based optical detection system that measures platelet-induced aggregation in citrated whole blood. Concomitant glycoprotein IIb/IIIa inhibitor, clopidogrel, dipyridamole, streptokinase, and non-steroidal anti-inflammatory drug (NSAID) therapy affect the assay results. Recent studies have used the RPFA system to study the association between aspirin resistance and cardiovascular risk (13,35).

Platelet activation. Platelet activation can also be measured by the release of arachidonic acid metabolites. Urinary levels of 11-dehydro TXB_2, a stable metabolite of TXA_2, have been used to study the extent of aspirin-mediated inhibition of TX generation (11). However, it is not known whether the persistent elevation in urinary TXB_2 levels is explained simply by uninhibited platelet COX-1 activity or perhaps also by COX-1 independent sources of TXA_2 generation. Serum markers, such as soluble CD40 ligand and P-selectin, have also shown promise as measurements of platelet activation (36). To date, however, little is known about these markers and their correlation with platelet aggregometry, especially in the context of aspirin therapy.

ASPIRIN RESISTANCE

Prevalence. The concept of therapeutic resistance originated in response to the fact that the immediate biological effects of aspirin are not uniform among all patients. Mehta et al. (37) demonstrated that a single 650-mg dose of aspirin produced minimal platelet inhibition in 30% of patients with coronary artery disease (CAD). Variability in aspirin-mediated platelet inhibition has subsequently been documented among normal subjects, in patients with cerebrovascular disease, stable CAD, and those presenting for coronary artery bypass surgery (7–9,38–44). 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 lead to a wide range of population estimates (Table 1).

Clinical relevance. The fact that biochemical measures of aspirin nonresponsiveness have been documented in a wide range of patient populations does not necessarily mean that it has a causal association with cardiovascular disease. Even if causal, the magnitude of risk associated with aspirin resistance may not be clinically important. A number of studies have attempted to address the uncertainties between platelet reactivity, aspirin therapy, and risk of future atherothrombotic events.

Clinical observations have suggested that the relationship between aspirin resistance and cardiovascular risk is in fact causal. Grundmann et al. (41) reported that, among patients with prior 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%). In another study, investigators reported that, among a population of high-risk patients taking daily aspirin therapy, the incidence of aspirin resistance was 23.4%, and individuals with a history of CAD had nearly twice the odds of being resistant (35).

Prospective studies have further validated the relationship between aspirin resistance and cardiovascular risk and have demonstrated that the magnitude of risk may not be trivial (Table 2). Grotemeyer et al. (42) reported a 30% incidence of aspirin resistance among post-stroke patients (as defined by a platelet reactivity index >1.25) after the ingestion of 500 mg aspirin. At two-year follow-up, the aspirin nonresponders had a 10-fold increase in the risk of recurrent vascular events as compared to aspirin-sensitive patients (7). Among patients with intermittent claudication who presented for a peripheral vascular angioplasty procedure, Mueller et al. (45) reported a 40% incidence of aspirin resistance. After 18 months of follow-up, aspirin resistance was associated with an 87% increase in the risk of arterial reocclusion. In a nested case-control study among aspirin-treated patients within the Heart Outcome Prevention Evaluation (HOPE) trial, investigators found that the risk of MI, stroke, or cardiovascular death increased with each increasing quartile of urinary 11-dehydro TXB_2. Those in the upper quartile had a 2-fold increase in risk of MI and 3.5-fold increase in risk of cardiovascular death when compared to those in the lowest quartile (11). Among 326 patients with stable CAD presenting for cardiac catheterization, Gum et al. (12) reported a 5% incidence of aspirin resistance, as defined by optical platelet aggregation. After a mean follow-up of 2.1 years, aspirin resistance was associated with a significant increase (hazard ratio 3.12, 95% confident interval 1.1 to 8.9) in the risk of MI, stroke, or death. More recently, Chen and colleagues (13) reported an association between aspirin resistance and creatinine kinase-MB elevation after nonurgent percutaneous coronary intervention procedures. In their population of 151 patients with stable CAD, the incidence of aspirin resistance, as defined by the Ultegra RPFA, was 19.2%. Despite adequate pretreatment with clopidogrel and procedural anticoagulation with heparin, aspirin resistance was associated with a 2.9-fold increased risk of creatinine kinase-MB elevation compared to aspirin-sensitive patients.

Observations from large randomized clinical trials involving patients with CAD who experience atherothrombotic events while on aspirin therapy also support the validity of aspirin resistance and suggest that the associated risk is not insignificant. 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 (46). Similarly, investigators from the PURSUIT trial reported that among 9,461 patients presenting with acute coronary syndromes, those previously taking aspirin were 20% more likely to suffer a recurrent event in the following six months as compared to patients who were previously aspirin-naive (47).

Potential mechanisms of aspirin resistance. The mechanism for aspirin resistance is uncertain. The answer is likely a combination of clinical, biological, and genetic properties affecting platelet function (Table 3). From the clinical perspective, behavioral habits (i.e., tobacco use), compliance with prescribed therapy, co-pharmacy (i.e., NSAIDs), and duration of aspirin therapy may help contribute to individual differences in aspirin responsiveness. Although tobacco use has been shown by some investigators to increase platelet activation and accentuate platelet thrombus formation despite aspirin-mediated suppression of TXA_2 synthesis (48), the scientific data in support of this finding has been inconsistent (49). Some investigators have reported that the increased risk of recurrent events in patients taking aspirin might be explained primarily by nonadherence to therapy. Cotter et al. (50) reported that, among 73 patients prescribed daily aspirin therapy after MI, the rate of adverse events (death, MI, or unstable angina) within 12 months was greater among patients considered nonadherent (42%) as compared to those considered adherent (6%) or biologically resistant (11%). The role of NSAIDs in attenuating the long-term antithrombotic benefits of aspirin has been reported but remains controversial (51,52). Nonselective NSAIDs have a strong binding affinity for a specific region of platelet COX-1 and may prevent aspirin-mediated acetylation and enzyme inhibition. However, current data are not consistent or definitive in proving the clinical relevance of this potential interaction. The duration of therapy may

Table 2. Adverse Clinical Outcomes Associated With Aspirin Resistance

Investigators	Study Population	Aspirin Resistance	Clinical Outcomes Associated With Aspirin Resistance
Grotemeyer et al. (7)	Cerebrovascular disease	30%	10-fold increased risk of MI, death, or stroke at two years
Eikelboom et al. (11)	High CVD risk	Quartiles of urinary TXB_2 levels	Highest TXB_2 quartile associated with a 2-fold increased risk of MI and 3.5-fold increased risk of death when compared to lowest quartile
Gum et al. (12)	Stable CAD	5.2%	3.1-fold increased risk of MI, death, or stroke at 1.8 years
Chen et al. (13)	Nonurgent PCI	19.2%	2.9-fold increased risk of significant CK-MB rise after PCI

CAD = coronary artery disease; CK = creatinine kinase; CVD = cardiovascular disease; MI = myocardial infarction; PCI = percutaneous coronary intervention; TX = thromboxane.

Table 3. Potential Mechanisms of Aspirin Resistance

Clinical
1) Noncompliance with prescribed aspirin therapy
2) Tobacco: enhanced platelet function
Pharmacodynamic
1) Dose-response: the antiplatelet or anti-inflammatory effects of aspirin might be dose-related
2) Duration of therapy: the biological response to aspirin might be reduced with long-term therapy
3) Drug interactions: NSAIDs might inhibit aspirin-mediated COX-1 acetylation and attenuate the long-term antithrombotic benefits of aspirin
Biological
1) Aspirin-insensitive TXA_2 biosynthesis: inducible COX-2, or regenerated COX-1, activity in macrophages and vascular endothelial cells may augment TXA_2 production
2) Alternate pathways for platelet activation:
a) Increased collagen sensitivity in aspirin nonresponders may lead to increased platelet adhesion
b) Failure to inhibit catecholamine-mediated (e.g., exercise, mental stress, epinephrine) platelet activation
c) Failure to inhibit non-TXA_2-mediated (e.g., adenosine diphosphate, platet-activating factor, and thrombin) platelet activation
3) Prostaglandin-like compounds: isoprostanes are produced from arachidonic acid and lipid peroxidation and may have properties similar to TXA_2
4) Vascular inflammation: increased platelet membrane expression of CD40 ligand may represent a novel pathway of platelet activation and/or a link to platelet-mediated participation in vascular inflammation
Genetic
1) Mutations and/or polymorphisms of the COX-1 gene: may prevent aspirin-mediated COX-1 acetylation
2) Glycoprotein IIb/IIIa receptor polymorphisms (PI^{A2})

COX = cyclooxygenase; NSAID = non-steroidal anti-inflammatory drug; TX = thromboxane.

also contribute to aspirin nonresponsiveness. Pulcinelli et al. (53) recently reported the effects of 2, 6, 12, and 24 months of aspirin treatment (100 or 330 mg/day) on ADP and collagen-induced platelet aggregation among 150 subjects with clinical evidence of atherothrombosis. Despite adequate platelet inhibitory response at two months, long-term treatment with aspirin was associated with progressive reduction in sensitivity to its effects. The investigators also reported, consistent with previous observations, that the sensitivity to aspirin was not dose-dependent.

The redundancy of platelet activation pathways and receptors may contribute to the problem of aspirin resistance. More specifically, pathways involving non–TXA_2-dependent activators such as, thrombin, ADP, epinephrine, and collagen can bypass the aspirin-mediated inhibitory effect leading to platelet activation and thrombosis. Catecholamine-induced platelet aggregation is one such pathway that might not be adequately inhibited by aspirin. Hurlen et al. (54) reported that, among patients with a previous MI, aspirin achieved adequate antiplatelet effects at rest but failed to inhibit exercise-induced increases in platelet aggregation. Similarly, aspirin pretreatment among healthy volunteers has been shown to attenuate basal platelet aggregability but not the effect of norepinephrine infusion on platelet aggregation (55). Both observations suggest that the antiplatelet effects of aspirin may be overcome during periods of increased sympathetic nervous system activity, such as during exercise or mental stress. Finally, there is evidence to suggest that aspirin nonresponders may have increased platelet sensitivity to collagen (56).

Alternate pathways for TXA_2 synthesis and the identification of prostaglandin-like compounds (isoprostanes) also provide potential explanations for the syndrome of aspirin resistance. Aspirin-insensitive TXA_2 biosynthesis can occur as a result of COX-2 induction in nonplatelet cells (monocytes/macrophages or endothelial cells) resulting from local inflammatory stimuli (20,57). These cells can both release TXA_2 or provide its precursor, PGH_2, to the aspirin-inhibited platelets. Additionally, COX-2 is present in newly formed platelets and may account for detectable levels of TXA_2 synthesis during periods of increased platelet turnover (19). Isoprostanes, resulting from lipid peroxidation, circulate at increased concentrations in patients with unstable angina, diabetes mellitus, hyperlipidemia, and in cigarette smokers. In addition, acting as vasoconstrictors, isoprostanes may have a role in amplifying the response of platelets to other agonists (18,58).

The interaction of platelets with other cells, such as erythrocytes or vascular endothelial cells, may also affect aspirin-mediated inhibition. It has been demonstrated, for example, that erythrocytes induce an increase in platelet TXB_2 synthesis and release of serotonin, beta-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 TXA_2 synthesis (59). As stated previously, the effect of aspirin-mediated inhibition of endothelium-derived PGI_2 production is not entirely known but may play a role in antagonizing the antithrombotic effects of aspirin at higher doses (17). The increased concentration of circulating platelet-monocyte aggregates during acute atherothrombotic events represents an accepted link between inflammation and thrombosis. There is growing evidence that CD40-CD40 ligand interactions might play an important role in both platelet activation, arterial thrombosis, and platelet-mediated pathways of vascular inflammation (36). To what extent platelet CD40 ligand expression and CD40-CD40 ligand interactions are inhibited by aspirin is in large part unknown.

Lastly, aspirin resistance might in part be explained by genetic differences in the COX-1 gene or the glycoprotein IIb/IIIa receptor complex. Polymorphisms of the IIIa subunit have been identified, and specific alleles, $Pl^{AI/A2}$ and $Pl^{A2/A2}$, are associated with increased thrombin formation and a lower threshold for platelet activation, alpha-granule release, and fibrinogen binding (Table 3). The antithrombotic effects of aspirin might be attenuated among carriers of the Pl^{A2} polymorphism (60,61). 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 (20).

FUTURE DIRECTIONS

Several studies have reported variable platelet response and potential therapeutic resistance to thienopyridines (62–65), and clopidogrel resistance has recently been linked to adverse clinical outcomes (65,66). However, similar to the study of aspirin resistance, there is no standardized definition of clopidogrel resistance, and further investigation is necessary to determine its population prevalence, clinical significance, and biological mechanism. Although less well-established, resistance to other antithrombotic therapies, such as glycoprotein IIb/IIIa antagonists, is biologically plausible and may in part explain some of the variability in patient response to these therapies.

CONCLUSIONS

Aspirin resistance remains broadly defined but appears to represent a valid and important biological phenomenon with significant clinical implications. There is substantial evidence to suggest that a significant percentage of individuals who take aspirin demonstrate resistance to its antithrombotic effects, as measured by one or more laboratory tests. Additionally, there is growing evidence that laboratory measures of aspirin nonresponsiveness may predict increased risk of future atherothrombotic events.

There are, however, many unanswered questions that need to be addressed before applying the concept of aspirin resistance to clinical practice and risk stratification. 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 strategy for affected individuals. Some of these questions may 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. Until then, aspirin remains a proven, and powerful, therapy against the atherothrombotic complications of cardiovascular disease.

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