Aspirin Resistance in Cardiovascular Disease: A Review of Prevalence, Mechanisms, and Clinical Significance

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Introduction

Salicylates have been used to treat pain and inflammation associated with rheumatism and other conditions since ancient times. The Assyrians initially described the use of willow leaves in treating rheumatism (1). This practice was continued into the 19th century when the first scientific studies evaluating this remedy were published in Europe. The active ingredient, salicylic acid, was first discovered in 1838 by the Italian chemist Raffaele Piria (1). Herman Kolbe of Marburg University elucidated the chemical structure and synthesized the compound in 1859 opening the door for industrial production of salicylic acid (1). However, early preparations of salicylic acid were plagued by side effects, such as unpleasant taste and dyspepsia, leading to discontinuation of treatment in a significant number of patients. This inspired Felix Hoffmann of Friedrich Bayer & Co. to develop a stable and better-tolerated form of the drug: acetylsalicylic acid. This new compound was marketed in 1899 as “Aspirin” (1). This acetylation step would prove to have consequences that reached well beyond tolerability and pain relief. In fact, it is now clear that it is responsible for aspirin’s widespread clinical use as an antiplatelet agent.

In the 1950’s, anecdotal reports linked aspirin use with prolongation of bleeding times (2). Subsequently, in the 1970’s, initial work on animal models began to elucidate the mechanism responsible for aspirin’s antithrombotic effect. As the importance of prostaglandins (especially thromboxane A2) in platelet activation and aggregation was becoming evident, Vane demonstrated that aspirin is a potent inhibitor of prostaglandin synthesis (3, 4). Majerus and Roth et al. were able to further characterize the interaction between aspirin and platelets (5, 8). They showed that low concentrations of aspirin irreversibly acetylate platelet cyclooxygenase, thereby blocking the formation of thromboxane A₂ for the lifetime of the platelet (5-8). Importantly, in the doses necessary to achieve platelet inhibition, aspirin did not inhibit endothelial cell prostaglandin synthesis, particularly prostacyclin (PGI₂), a potent vasodilator (9–11). It was later confirmed that aspirin acetylates serine-530 in the active site of the cyclooxygenase-1 enzyme (prostaglandin H₂ synthase-1), permanently deactivating it and preventing thromboxane A₂ platelet activation (12, 13). These observations paved the way for the clinical investigation of aspirin’s antiplatelet effects in preventing thrombotic events, such as ischemic strokes and acute myocardial infarctions.

Aspirin’s use for the secondary prevention of vascular events is well established. Recently, the Antithrombotic Trialists’ Collaboration compiled a meta-analysis of 65 trials using aspirin in high-risk patients and found a 23% odds reduction in vascular events in the aspirin-treated groups (14). Aspirin is also a very effective therapy for patients suffering an acute myocardial infarction. As demonstrated by the Second International Study of Infarct Survival (ISIS-2) trial, acute aspirin administration reduced mortality by 23%, a comparable (and importantly additive) effect to thrombolytic therapy (15).

Aspirin also has an important role in primary prevention of cardiovascular events. The Physician’s Health Study demonstrated a 44% reduction in the incidence of a first myocardial infarction in middle aged men treated with aspirin compared with placebo over a 5 year follow-up period (16–17). Although data for the use of aspirin as primary prevention for cardiovascular events in women are currently lacking, physicians routinely recommend aspirin therapy both for women with known atherosclerotic vascular disease, but also for those at high risk for future events.

Identifying the Problem of Aspirin Resistance

As evident by the aforementioned trials, aspirin is very effective for both the primary and secondary prevention of thrombotic atherosclerotic events; however, there are still patients who suffer “breakthrough” events despite daily aspirin therapy. In addition to these clinical observations, studies examining platelet aggregation after aspirin treatment have indeed demonstrated wide variability in its antiplatelet effects among patients (Table 1). It is based on this constellation of clinical and laboratory evidence of a diminished or absent response to aspirin in some individuals, that the concept of “aspirin resistance” was generated.

Initial evidence that some patients may be resistant to aspirin’s antiplatelet action came from a study by Mehta et al. who showed that 30% of patients with coronary artery disease had minimal inhibition of platelet aggregation after a single 650 mg dose of aspirin (18). Similarly, Buchanan et al. found that bleeding times were prolonged in only 23 of 40 patients presenting for elective coronary bypass surgery taking 325 mg of aspirin daily, again suggesting an approximately 40% prevalence of aspirin resistance (19). Using 143 patients enrolled in the Warfarin-Aspirin Reinfarction Study (WARIS-II), Hurlen et al. noted that platelet aggregation was not inhibited in 14 patients on daily aspirin, but this was overcome in all but 2 patients by an additional 75 mg or 160 mg oral dose (20). This wide variability in the antiplatelet effects of aspirin is not only present in patients with coronary artery
disease, but has also been demonstrated among young, healthy volunteers (19, 21).

Does such a diminished response to aspirin or even frank resistance to its antiplatelet effects have clinical significance? There are clinical studies suggesting that aspirin resistance is indeed clinically important. First, in a study of post-stroke patients, aspirin resistance, defined as normal platelet function a few hours after aspirin administration, was present in 30% of patients (22). Importantly, 2 year follow-up of these patients showed an 89% increase in the risk for a subsequent vascular event among aspirin resistant patients compared with aspirin responders (23). Similar adverse outcomes have been demonstrated in patients with peripheral vascular disease. Mueller et al. reported that among 100 patients undergoing peripheral arterial angioplasty only 40% demonstrated appropriate platelet inhibition after 100 mg of aspirin (24). Importantly, aspirin nonresponders had an 87% increase in the risk of arterial re-occlusion during follow-up (24). Adverse outcomes have also been demonstrated in patients with coronary artery disease. It appears, however, that there is a substantial number of patients who are not getting the intended anti-platelet effect of daily aspirin therapy.

### Measuring Platelet Function

Traditionally, platelet function has been assessed using platelet aggregation in an optical aggregometer. In this test, light transmittance is measured through platelet-rich plasma with platelet poor plasma used as an optical blank. A platelet agonist is added to the platelet rich plasma and light transmission is determined and reported on a 0-100% scale. Increased platelet aggregation results in increased light transmittance. Although useful in investigational studies, this test is labor-intensive and its reproducibility depends on platelet concentration, agonist type and concentration, as well as the end-points used (28). Therefore, it is not easily adapted for use by the practicing clinician.

Recently, simpler, more rapid tests of platelet function have been developed. Whole blood aggregometry eliminates the step of preparing platelet-rich plasma. The assay measures electrical impedance between two electrodes in a sample of whole blood. After agonist is added, platelet aggregates build up on the electrodes and increase impedance. This test has been used in the clinical setting, but results have not correlated well with optical aggregometry in glycoprotein IIb/IIIa inhibitor testing (28). Finally, assessment of bleeding time has been used successfully as a measure of platelet response to aspirin (19), however, its clinical use has been limited since it is operator dependent, associated with patient discomfort, and can leave a small, permanent scar.

Currently the most appealing test for assessment of platelet inhibition in clinical practice appears to be the PFA-100® (Dade Behring) 9.5% were aspirin resistant (27). Currently, there are no prospective studies specifically correlating suboptimal responses to aspirin with adverse outcomes in patients with cardiovascular disease. It appears, however, that there is a substantial number of patients who are not getting the intended anti-platelet effect of daily aspirin therapy.

### Table 1: Evidence for Aspirin Resistance

<table>
<thead>
<tr>
<th>Population Studied</th>
<th>ASA dose (mg/day)</th>
<th>Method</th>
<th>Criteria for ASA Resistance</th>
<th>% ASA Resistance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CABG Patients (n=40)</td>
<td>325</td>
<td>Bleeding Time</td>
<td>No prolongation of bleeding time above baseline</td>
<td>43%</td>
<td>19</td>
</tr>
<tr>
<td>AMI Patients (n=143)</td>
<td>75-160</td>
<td>Platelet Aggregation Ratio (PAR)</td>
<td>PAR ≤ 0.82 after ASA</td>
<td>9.8%</td>
<td>20</td>
</tr>
<tr>
<td>Healthy young adults (n=31)</td>
<td>325</td>
<td>Whole Blood Assay: samples incubated with arachidonic acid until aggregation occurred</td>
<td>Aggregation time before and after ASA. Mean response after ASA was doubling of aggregation time, but a highly variable response seen</td>
<td>Not Determined</td>
<td>21</td>
</tr>
<tr>
<td>Stroke Patients (n=180)</td>
<td>500</td>
<td>Platelet Reactivity (PR): aggregation induced by blood collection</td>
<td>Normal PR Index (&lt; 1.25) at 2 or 12 hours = resistance PR index &gt; 1.25 at 2 and 12 hours = expected response</td>
<td>36%</td>
<td>22</td>
</tr>
<tr>
<td>PVD Patients (n=100)</td>
<td>100</td>
<td>Corrected Whole Blood Aggregation using ADP and collagen agonists.</td>
<td>Platelet aggregation after agonist compared to baseline values (&gt;40% of baseline after ASA dose was considered resistance)</td>
<td>60%</td>
<td>24</td>
</tr>
<tr>
<td>Patients With Stable CAD (n=325)</td>
<td>325</td>
<td>Optical Platelet Aggregation by ADP and arachidionic acid</td>
<td>Normal ADP induced aggregation and arachidonic acid induced &gt;20% after ASA = resistance</td>
<td>5.5%</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PFA-100® using collagen/ADP and collagen/EP</td>
<td>PFA-100®: Normal (&lt;193s) collagen/EPi closure time after ASA = resistance</td>
<td>9.5%</td>
<td></td>
</tr>
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through the aperture by a vacuum to simulate shear stress on the platelets. A platelet plug forms and occludes the aperture. Aperture closure time is a measure of platelet activity. The results are readily reproducible and correlate well with optical aggregometry results (27, 28). This test has been studied as a monitor of platelet response to aspirin therapy, and has proved to be a useful test for monitoring the pharmacological effects of aspirin and identifying aspirin resistant patients (27, 29).

**Possible Mechanisms of Aspirin Resistance**

Although much is currently known about aspirin’s effect on platelets, the mechanism by which some patients’ platelets are resistant to this effect has not been established. It has been shown that aspirin uniformly acetylates and inhibits cyclo-oxygenase-1 at very low concentrations (5-8). In some individuals, however, this inhibitory effect is somehow circumvented, allowing for normal platelet function despite aspirin therapy (Table 2) (18-27).

There are a number of extrinsic factors that can modify aspirin’s ability to inactivate platelets. Enhanced platelet activation may “override” aspirin’s effect. Cigarette smoking has been shown to accentuate platelet thrombosis in a way that is not inhibited by aspirin (30), however recent studies show that patients with aspirin resistance are less likely to be smokers (27). In the early 1980’s, it was shown that the non-steroidal anti-inflammatory drugs (NSAID’s) ibuprofen and indomethacin block the long-lasting antiplatelet effects of aspirin (31, 32). Recent reports demonstrate that these NSAID’s bind to the active site of cyclooxygenase-1, thereby blocking aspirin’s access to Ser-530 and preventing acetylation (33, 34). It has not been determined, however, how this biochemical interaction effects aspirin’s clinical cardio-protective effects. Potentially, such an interaction may lead to a secondary aspirin resistance in patients who are usually aspirin responders. This does not, however, fully account for the lack of response to aspirin seen in recent studies, since NSAID use was an exclusion (19, 21, 24, 27).

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<th>Table 2 Proposed mechanisms of aspirin resistance</th>
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**I. Extrinsic Mechanisms**

A. Accentuation of platelet thrombosis by exogenous substances (i.e. cigarette smoke). 30

B. Drugs, such as nonsteroidal anti-inflammatory drugs, that may interact with aspirin’s acetylation of cyclooxygenase-1. 31-34

C. Increased platelet turnover overcoming once-daily aspirin dosing 35

D. Inadequate aspirin dosing. 36, 37

**II. Intrinsic Mechanisms**

A. Inducible cyclooxygenase-2 that is not adequately inhibited by low-dose aspirin, thereby allowing for platelet thromboxane A2 production despite inhibition of cyclooxygenase-1. 41

B. Polymorphisms in the cyclooxygenase-1 gene that alter the structure of the active site and prevent acetylation by aspirin. 36, 43

C. Regenerated, uninhibited cyclooxygenase-1 in nucleated cells such as macrophages and vascular endothelial cells producing prostaglandin H2 that is shunted into platelets, thereby bypassing platelet cyclooxygenase-1. 36, 43

D. Polymorphisms in the glycoprotein IIb/IIIa receptor complex that confers varying degrees of platelet responsiveness to aspirin. 48-51

It is also possible that increased platelet turnover could explain a lack of platelet inhibition by once-daily aspirin dosing. Zimmerman et al. proposed that post coronary bypass patients with increased platelet turnover could make a significant number of new, active platelets after the daily aspirin dose has cleared (35). In accordance, they found that thromboxane production was only 30-50% inhibited in post-bypass surgery patients receiving daily aspirin as compared to 94% inhibition in healthy volunteers on the same regimen (35). Although this effect appears significant in the immediate post-bypass period, it is unlikely that non-surgical patients have sufficiently increased platelet turnover to overcome daily aspirin dosing.

Although low-dose aspirin is expected to inhibit cyclooxygenase-1 completely (5-8), some patients may require higher doses to achieve the desired antiplatelet effect. This idea has been explored in the stroke literature in the early 1990’s. Helgason et al. showed that escalating the dose of aspirin to as high as 1300 mg did produce complete inhibition of platelet aggregation in 25 of 28 stroke patients who had only a partial response to lower doses (36). This suggests that there is a dose response curve for aspirin’s ability to inhibit platelet function. Up to 8% of patients, however, appeared to be resistant even at 1300 mg daily doses (37). Although no ideal aspirin dose for prevention of vascular events has been established, escalating the dose is not practical in many patients secondary to gastrointestinal side-effects. Furthermore, the same study demonstrated that a constant dose of aspirin produces variable platelet inhibition over time (37), indicating that temporal fluctuations in individual patients may contribute to the variable response to aspirin seen in some studies.

It is also possible that an intrinsic mechanism within the platelet itself explains aspirin resistance. In this scenario, resistant platelets can produce thromboxane A2 despite aspirin therapy. Cyclooxygenase-1 is responsible for thromboxane formation in platelets and is expressed in most cells in the body. Cyclooxygenase-2 is normally undetectable, but is inducible in many tissues (38, 39). Aspirin inhibits cyclooxygenase-1.
166-times more potently than cyclooxygenase-2 (40). Since platelets have no nuclear structures, it has long been thought that once cyclooxygenase-1 is irreversibly inhibited, thromboxane synthesis is blocked. Recent evidence, however, has shown that platelets do contain cyclooxygenase-2 mRNA (41). Therefore, this enzyme may be inducible in platelets under stress (41). Since low-dose aspirin is an ineffective inhibitor of cyclooxygenase-2, this mechanism could provide an alternate pathway for thromboxane production in aspirin treated platelets. This concept has been challenged, however, by Patrignani et al. who found no evidence of inducible cyclooxygenase-2 enzyme in platelets of healthy volunteers (42). Nevertheless, thromboxane production is elevated in some patients on daily aspirin (26). One proposed explanation for this is a potential polymorphism of cyclooxygenase-1 which could confer resistance to the acetylation of Ser-530 by aspirin. (26, 43). Another is the production of prostaglandin H₂ by regenerated cyclooxygenase-1 in nucleated cells, such as macrophages or vascular endothelial cells, which can be shunted to platelets allowing for production of thromboxane A₂ without platelet cyclooxygenase-1 (26, 43).

Genetic differences in the glycoprotein IIb/IIIa receptor complex may also be responsible for aspirin’s variable effects in different patients. The glycoprotein IIb/IIIa receptor is the final common pathway for platelet activation, and a frequent polymorphism involving the substitution of Leu33 (PFA1) to Pro33 (PFA2) is known (44). In central Europeans, the PFA1A2 allele is present in 20-30% of people and the PFA2A2 allele is present in 1-3% of people (45). It has been shown that platelets containing PFA1A2 or PFA2A2 alleles are more reactive than homozygous PFA1A1 platelets with enhanced thrombin formation and a lower threshold for activation, α-granule release, and fibrinogen binding (46, 47). Although evidence is conflicting (48), most studies indicate that PFA2 carriers are less responsive to the antithrombotic effects of aspirin (46, 47, 49). Studies implicating the PFA2 allele as a risk factor for coronary artery disease have been inconclusive (50-52) and no studies have correlated the presence of the PFA2 allele and aspirin resistance in the general population. It is likely that there are yet additional, unidentified genetic factors contributing to intrinsic aspirin resistance. Further work needs to be done to elucidate these mechanisms as this could guide future strategies for identifying and managing aspirin resistant patients.

The Future of Antiplatelet Therapy

Low-dose daily aspirin is clearly a useful therapy for primary and secondary prevention of cardiovascular events (14, 16, 17). It also has proven benefit if administered during acute events (15). Aspirin is easy to give, inexpensive, and has relatively few side effects at low doses. Therefore, aspirin is unlikely to ever be replaced as a first-line antiplatelet agent. As newer agents that work by different mechanisms become available, however, one must explore how these agents can be used to maximize patients’ benefit from antiplatelet therapy. Data from the Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) trial demonstrate that patients treated with clopidogrel instead of aspirin have a 7-8% relative risk reduction in vascular events (53). Recently, the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial showed that patients with acute non-ST-segment elevation myocardial infarctions and unstable angina treated with aspirin and clopidogrel within 24 h of presentation had a 20% relative risk reduction in vascular events compared with aspirin alone (54).

Identifying which patients would benefit most from antiplatelet therapies such as clopidogrel in addition to aspirin is the next challenge. There is currently no standard definition for the identification of such “aspirin resistant” patients. Although for the purpose of clinical practice recurrent events while on aspirin therapy may be sufficient grounds for combination antiplatelet therapy, we propose that accurate laboratory identification of these individuals using standardized criteria should be the optimal practice in the future. Previous studies have employed a variety of methods of measuring platelet function (Table 1). Most of these are complicated and not suitable for widespread clinical use. Recently, though, point-of-care tests such as the PFA-100⁰ have become available. Such methods could be used in the clinical setting to measure patients’ response to aspirin and identify aspirin resistance. Consequently, individualized, targeted aspirin dosing and, when necessary, combination therapy with agents such as clopidogrel, could be used to achieve the desired antiplatelet effect with maximal clinical benefit and minimal side effects.

Obviously much work needs to be done before this idea can become the standard of care. Does aspirin response predict clinical outcomes? Do aspirin resistant patients have less vascular events on alternative or additional antiplatelet therapy? Does individualization of antiplatelet therapy improve risk/benefit ratios? These are but a few of the questions yet to be answered. Ideally, well-planned, randomized clinical trials utilizing a standard definition of aspirin resistance will answer these questions, ushering a new era of improved prevention and treatment of cardiovascular disease with antiplatelet therapies.

References

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