Editorials

Aspirin Resistance

EFTHYMIOS N. DELIARGYRIS, HARISIOS BOUDOULAS

Foundation of Biomedical Research, Academy of Athens, Athens, Greece

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Corresponding Author:
Efthymios N. Deliargyris

5-7 Distomou St., Marousi, 15125, Athens, Greece
e-mail:
edeliargyr@aol.com

The use of salicylates to treat pain and inflammation associated with rheumatism and other conditions can be traced to ancient times. Early preparations of salicylic acid were plagued by side effects, but in the late 1800's Felix Hoffmann of Friedrich Bayer & Co. developed a more stable and better-tolerated form of the drug, named acetylsalicylic acid. This new compound was marketed in 1899 as "Aspirin"[1]. In the mid 1900's the initial reports linking aspirin use with prolongation of bleeding times emerged[2]. We now know that aspirin acetylates serine-530 in the active site of the cyclooxygenase-1 enzyme (prostaglandin H_2 synthase-1), thereby permanently deactivating it and preventing thromboxane A_2 platelet activation[3-5].

Aspirin's ability to prevent 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[6]. Aspirin is also a very effective therapy for patients suffering an acute myocardial infarction, where early administration can reduce mortality by 23%, a comparable (and importantly additive) effect to thrombolytic therapy[7]. Further, for the primary prevention of cardiovascular events, the Physician's Health Study demonstrated a significant reduction in the incidence of a first myocardial infarction in middle aged physicians treated with aspirin compared with placebo over a 5 year follow-up period[8,9]. Such data have supported our everyday clinical practice of prescribing aspirin in all patients with or at risk for atherosclerotic vascular disease.

Evidence for aspirin resistance

We all know that despite daily aspirin therapy some patients will still experience "breakthrough" events. Laboratory studies examining platelet aggregation after aspirin treatment have also demonstrated wide variability in its antiplatelet effects among patients. The concept of "aspirin resistance" is based on this constellation of clinical and laboratory evidence of a variable response to aspirin. A diminished or absent antiplatelet effect to aspirin has been demonstrated in patients undergoing coronary bypass surgery, myocardial infarction survivors and even among healthy, young volunteers (Table 1)[10-13].

Several studies have suggested that aspirin resistance is clinically important. Thus, aspirin resistance was present in 30% - 40% of stroke or peripheral vascular disease patients and was associated with a >80% increase in the risk for a repeat vascular event during a 2 year follow-up period compared to patients without aspirin resistance[14-16]. In the randomized, prospective Platelet IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy (PURSUIT) trial of 9,461 patients presenting with non-ST segment

Table 1. Evidence for aspirin resistance.

Population Studied	ASA dose (mg/day)	Method	Criteria for ASA Resistance	% ASA Resistance	Ref.
CABG patients (n=40)	325	Bleeding Time	No prolongation of bleeding time above baseline	43%	19
AMI patients (n=143)	75-160	Platelet Aggregation Ratio (PAR)	PAR $\leq$ 0.82 after ASA	9.8%	20
			PAR $\leq$ 0.82 after additional ASA	1.4%	
Healthy young adults (n=31)	325	Whole Blood Assay: samples incubated with arachidonic acid until aggregation occurred	Aggregation time before and after ASA. Mean response after ASA was doubling of aggregation time, but a highly variable response seen	Not Determined	21
Stroke patients (n=180)	500	Platelet Reactivity (PR): aggregation induced by blood collection	Normal PR Index (< 1.25) at 2 or 12 hours = resistance PR index > 1.25 at 2 and 12 hours = expected response	36%	22
PVD patients (n=100)	100	Corrected Whole Blood Aggregation using ADP and collagen agonists.	Platelet aggregation after agonist compared to baseline values (>40% of baseline after ASA dose was considered resistance)	60%	24
Patients with stable CAD (n=325)	325	Optical Platelet Aggregation by ADP and arachidonic acid	Normal ADP induced aggregation and arachidonic acid induced >20% after ASA = resistance	5.5%	27
		PFA –100® using collagen/ADP and collagen/ EPI	PFA-100®: Normal (<193s) collagen/EPI closure time after ASA = resistance	9.5%	
Normal blood In vitro studies	0.01-100 mmol/L	Platelet aggregation	Platelet aggregation	Aspirin less effective on Pl^{A1}/Pl^{A1}	31

CABG=coronary artery bypass grafting, AMI=acute myocardial infarction CAD=coronary artery disease, PVD=peripheral vascular disease

elevation acute coronary syndromes, patients previously on aspirin were 20% more likely to suffer a recurrent event in 6 months compared with patients not previously on aspirin suggesting that aspirin had a lesser or no effect in this group of patients[17]. Finally, a recent case-control sub-study from the population of the Heart Outcomes Prevention Evaluation (HOPE) trial found that among aspirin-treated patients, those with higher concentrations of urinary 11-dehydro thromboxane B_2 (a stable metabolite of thromboxane A_2 suggesting incomplete inhibition) had a 2-times-higher risk of myocardial infarction and a 3.5-times-higher risk of cardiovascular death compared to those without urinary 11-dehydro thromboxane B_2 elevations.[18] It is important to note, however, that to date there are no prospective studies specifically correlating a sub-optimal response to aspirin with adverse outcomes in patients with cardiovascular disease.

Possible mechanisms of aspirin resistance

There are a number of extrinsic factors that may enhance platelet activation thereby enabling them to "override" aspirin's effect. Cigarette smoking has been shown to accentuate platelet thrombosis in a way that is not inhibited by aspirin[19]. Non-steroidal anti-inflammatory drugs such as ibuprofen and indomethacin may also interfere with the long-lasting antiplatelet effects of aspirin[20,21]. Increased platelet turnover such as that commonly seen after coronary bypass surgery may also diminish the response to aspirin through the production of a significant number of new, active platelets after the daily aspirin dose has cleared[22].

It is also possible that an intrinsic mechanism within the platelet itself may explain aspirin resistance, where resistant platelets can still produce thromboxane A_2 despite aspirin therapy. Cyclooxygenase-1 is responsible for thromboxane formation in platelets

and is also expressed in most cells in the body. Cyclooxygenase-2 is normally undetectable in platelets, but may be present in many other tissues in the body. Aspirin inhibits cyclooxygenase-1 166 times more potently than cycloxygenase-2. Platelets have no nuclear structures and when cyclooxygenase-1 is irreversible inhibited by aspirin, thromboxane synthesis is blocked[23]. Recent evidence, however, has shown that platelets do contain cyclooxygenase-2 mRNA. Thus, cyclooxygenase-2 which is not blocked by aspirin may be an alternate pathway for thromboxane production in aspirin treated platelets[24]. This concept has been challenged by others[25].

By definition, resistance to a pharmacological agent exists when at "therapeutic" drug concentrations there is no drug effect or less effect to that expected. In all studies related to aspirin resistance, however, aspirin plasma concentrations are not available. Thus, it is not certain if it is resistance to aspirin or simply inadequate aspirin concentrations.

Another plausible explanation for the phenomenon of aspirin resistance may be the inherited polymorphisms of the glycoprotein IIb/IIIa receptor complex, the final common pathway for platelet aggregation. The gene encoding the IIIa subunit of this receptor is located on chromosome 17 in adjacent locus to the ACE gene, where a cytosine to thymine substitution in position 1565 causes a leukine to proline substitution of the polypeptidic chain of the receptor (Pl^{A1}/Pl^{A2} polymorphism)[26,27]. In central Europeans, the $Pl^{A1/A2}$ allele is present in 20-30 % of people and the $Pl^{A2/A2}$ allele is present in 1-3 % of people.[28] It has been shown that platelets containing $Pl^{A1/A2}$ or $Pl^{A2/A2}$ alleles are more reactive than homozygous $Pl^{A1/A1}$ platelets with enhanced thrombin formation and a lower threshold for activation, granule release, and fibrinogen binding and therefore a variable response to the antiplatelet effects of aspirin[29,30]. Although evidence is conflicting[31-34], the recent Copenhagen City Heart Study of over 9,000 subjects with a follow-up of 22 years demonstrated that men, not women, less than age 50 with Pl^{A2}/Pl^{A2} polymorphism had up to 4-fold increase in ischemic cardiac events[35]. An effect of estrogen on Pl^{A2} polymorphism in premenopausal women is the most likely explanation for the gender differences in their findings. Further evidence for the role of GP IIIa polymorhisms in the variable antiplatelet responses to treatment comes from Boudoulas et al, who evaluated the effect of the PI^{A2} polymorhism on the response to estrogens. In this study, the effect of estrogen on platelet aggregation was highly variable and dependent on the presence of the PI^{A2} polymorhism[36]. This observation may represent the first real insight in the conflicting results of clinical trials evaluating the effects of hormone replacement therapy on cardiovascular events. It is likely that there are yet additional, unidentified genetic factors contributing to intrinsic aspirin resistance.

The future of antiplatelet therapy

Aspirin is easy to give, inexpensive, and has relatively few side effects at low doses and in such it 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[37]. 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 hours of presentation had a 20% relative risk reduction in vascular events compared with aspirin alone[38].

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 and our decisions should be based on the clinical evidence of recurrent events while on aspirin to define the need for combination antiplatelet therapy. Recently, though, point-of-care tests such as the PFA-100® have become available that allow rapid assessment of platelet function using whole blood and can be used at the bedside. The assay uses a cartridge that contains a small aperture coated with collagen and epinephrine or ADP. Blood in the cartridge is pulled through the aperture by a vacuum to simulate shear stress on the platelets. A platelet plug forms and occludes the aperture and the time to closure is a measure of platelet activity. The results of this technique correlate well with optical aggregometry and may represent a clinically useful way of identifying "aspirin-resistant" patients[39].

In the future, individualized aspirin dosing and, when necessary, combination therapy with agents such as clopidogrel, guided by platelet function testing,

could be used to achieve the desired antiplatelet effect with maximal clinical benefit and minimal side effects. It is quite possible that in the near future genetic analysis will be used to guide antiplatelet therapy. The hypothesis that aspirin is better in patients with Pl^{A1}/Pl^{A2} polymorphism, while clopidogrel is better in patients with Pl^{A1}/Pl^{A1} polymorphism is now tested in ongoing studies of the Ohio State University Medical Center supported by NIH.

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