Aspirin Resistance

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A B S T R A C T

Despite the development of new molecules, aspirin remains a mainstay of the antiplatelet therapy, indispensable for treatment and the secondary prevention of cardiovascular and cerebrovascular diseases. The antiplatelet effect of aspirin is mostly mediated through irreversible cyclooxygenase-1 inhibition resulting in the suppression of thromboxane A2 synthesis. However, the inter- and intraindividual variability of its antiplatelet effect is well known. Aspirin resistance can be understood from the clinical point of view--as a failure of the protective effect of aspirin from thrombotic complication or can be defined from the laboratory aspect. Laboratory diagnosis of aspirin resistance is based on the demonstration of the insufficient inhibition of platelet aggregation or the incomplete suppression of thromboxane A2 synthesis (assay for its metabolite in urine, 11-dehydrothromboxane). The prevalence of aspirin resistance varies from 5-45%, depending up on the platelet aggregation method used and the population studied. This prevalence of aspirin resistance is very important and of great alarming clinical importance. Many hypotheses have been put forward regarding the pathogenesis of aspirin resistance. Currently, there is no standard protocol for the management of these patients. Newer antiplatelet agents or the combination of these agents are likely to overcome this important clinical problem in future.

INTRODUCTION

It has been slightly more than 100 years since acetyl salicylic acid, the most widely consumed drug in the world has been synthesized, developed and commercialized. The first report of a possible antithrombotic effect of aspirin appeared in 1953.[1] This is followed by the discovery by numerous investigators that aspirin could significantly decrease platelet function.[2] Clinical trials have shown that aspirin is effective for both primary and secondary prevention of myocardial infarction (MI), stroke and cardiovascular death and in the management of MI, unstable angina and embolic stroke. 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 in all-cause mortality.[3] However a significant number of these patients manifest breakthrough events despite regular intake of aspirin. It is estimated that one in eight high risk patients suffers from the recurrence of a vascular event within the next 2 years. Based on the clinical and platelet function studies concept of aspirin resistance was described.

MECHANISMS OF ACTION OF ASPIRIN

Aspirin mediates its antithrombotic effect through inhibition of platelet aggregation. It does this by inhibition of cyclooxygenase-1 (COX-1) which in turn inhibits the metabolism of arachidonic acid to cyclic prostanoids such as thromboxane A2, prostacycline and other prostaglandins. 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).[4] Recently Cipollone et al[5] demonstrated that COX-2 is present in newly formed platelets (8-10% of circulating platelets) and that PGE-2 is the main product of platelet COX-2 activity. Aspirin is 170 times more potent in inhibiting COX-1 than COX-2.

DEFINITION OF ASPIRIN RESISTANCE

Aspirin resistance is a poorly defined term. It could mean a clinical inability of aspirin to protect individuals from arterial thrombotic events or laboratory indication of failure of aspirin to inhibit platelet activity, mainly platelet aggregation or a close to normal urinary concentration of thromboxane metabolites. There are no standard criteria or method by which aspirin resistance can be assessed.

PREVALENCE OF ASPIRIN RESISTANCE

Initial evidence that some patients may be resistant to aspirin came from a study by Metha et al [6] who showed that 30% of patients had minimal inhibition of platelet aggregation after a single 150 mg dose of aspirin. Subsequent studies attempted

Table 1 : Selected Studies Reporting the Prevalence of Aspirin Resistance

Investigators	No of pts.	Aspirin Dose (mg/d)	Methodology of Aspirin Res(%)	Prevalence (%)
Grotemeyer et al[7]	180 (post-stroke)	100	Platelet reactivity: aggregation induced by blood collection	36
Buchanan et al[8]	40 (CABG)	325	Bleeding time	43
Gum et al[11]	325 (stable CAD)	325	1. Optical platelet aggregation: ADP and arachidonic acid	5.5
			2. PFA-100 (collagen/ADP and collagen/epinephrine)	9.5
Chen et al[12]	151 (elective PCI)	80-325	RPFA: defined ASA resistance as ARU > 550	19.2
Helgason et al[13]	306 (post-stroke)	325	Optical platelet aggregometry using, ADP, arachidonic acid epinephrine and collagen	25
Macchi et al[14]	72 (stable CAD)	160	PFA-100: defined ASA resistance as epinephrine closure time <186sec	29.2
Andersen et al[15]	129 (stable CAD)	Aspirin (160) alone Aspirin (75) plus Coumadin	PFA-100: defined ASA resistance as epinephrine closure time < 196 sec	1.35 2.4
Wang et al[16]	422 (stable CAD)	325	RPFA: defined ASA resistance as ARU > 550	23.0
Sibi et al[17]	75 (stable CAD)	150	Optical platelet aggregation using arachidonic acid, ADP	26

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.

to estimate the prevalence of aspirin resistance in patients with cerebrovascular disease[7] and coronary artery disease.[8] Recently, Grundmann et al [9] 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%). Grotemeyer et al [7] reported a 30% incidence of aspirin resistance among post-stroke patients after the ingestion of 500 mg aspirin. Similarly, aspirin resistance was found in 40% of patients with intermittent claudication who presented for a peripheral vascular angioplasty procedure.[10] More recently, Gum and coworkers[11] reported a 5% incidence of aspirin resistance, as defined by optical platelet aggregation, among 326 patients with ischemic heart disease. Chen et al [12] reported a 19.2% incidence of aspirin resistance, as defined by the Ultegra RPFA, among 151 patients with coronary artery disease. The overall prevalence of aspirin resistance in different studies varied from 8% to 45% (Table-1).[7,8,11-17]

We did a prospective study to determine the prevalence of aspirin resistance in Indian patients with coronary artery disease and to look for any predictors for aspirin resistance. Patients who were taking 150 mg of aspirin for at least 7 days for secondary prophylaxis were included. Blood samples were drawn 1 to 24 hrs after administration of last dose of aspirin. Platelet aggregation study was done using PACKS4-aggregometer. Aspirin resistance was defined as a mean aggregation of more than 70% with ADP and more than 20% with arachidonic acid. Patients meeting only one of these criteria were defined as aspirin semi-responders. 75 patients were enrolled. There were 48 males and 27 females. 26% of patients with cardiac disease on aspirin were found to have some form of aspirin resistance (9.3% were aspirin resistant 17.3% were aspirin-semi-responders) (Fig.1 and 2). There were statistically significant correlation of aspirin-resistance with the presence of diabetes, systemic hypertension and dyslipidemia. But, history of cigarette smoking did not show any significant association with aspirin resistance.[17]

METHODS OF ASSESSING ASPIRIN RESISTANCE

1. Platelet aggregation studies using platelet rich plasma (optical aggregometer) or whole blood (platelet function analyzer-100; PFA-100) are the usually employed method. Optical aggregometer is widely available, however it is neither reproducible nor user-friendly. Whereas whole blood aggregometer allows more rapid assessment and results are easily reproducible.[18] Other method used is rapid platelet function analysis (RPFA) using Ultegra RPFA instrument.

2. Bleeding time[8]

3. Urinary metabolites of thromboxane metabolism- urinary 11-dehdrothromboxane B2 levels, a stable metabolite of TXA2.[19] As the urinary levels depend on platelet and non-platelet sources of thromboxane generation, this test lacks specificity.

4. Selectin-P overexpression.[20]

Criteria used for the diagnosis of aspirin resistance also varies among different authors. Using platelet aggregation Gum et al[11] defined resistance as aggregation of >70% with 10μm ADP and >20% with 0.5mg/ml arachidonic acid. Aspirin semiresponder was defined as meeting one criteria but not both. With PFA-100 aspirin resistance was defined in terms of normal collagen and/or epinephrine closure time <186 sec and with RPFA- as aspirin resistance units >550.[12]

MECHANISMS OF ASPIRIN RESISTANCE

The possible mechanisms of aspirin resistance are poorly understood.

Clinical

1. If the patient is noncompliant naturally there will be no benefit. Measurement of salicylate levels will help to address this issue. Many studies have focused on connecting aspirin resistance with inadequate dose response.[13,21] However, clinical outcome has not been shown to be affected by the
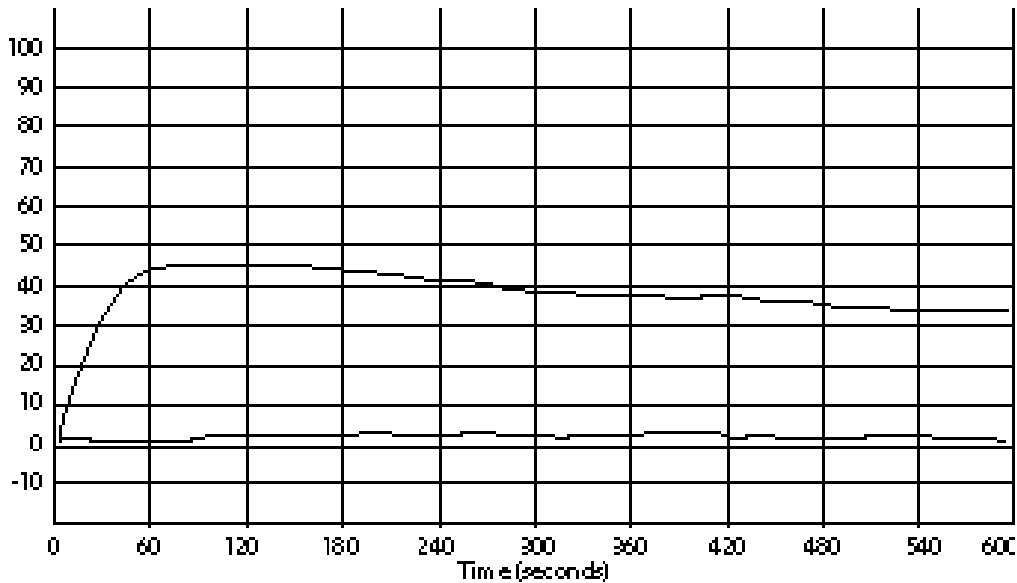
Fig. 1: Showing aspirin sensitivity- Aggregation curves are below 70% with ADP and below 20% with arachidonic acid
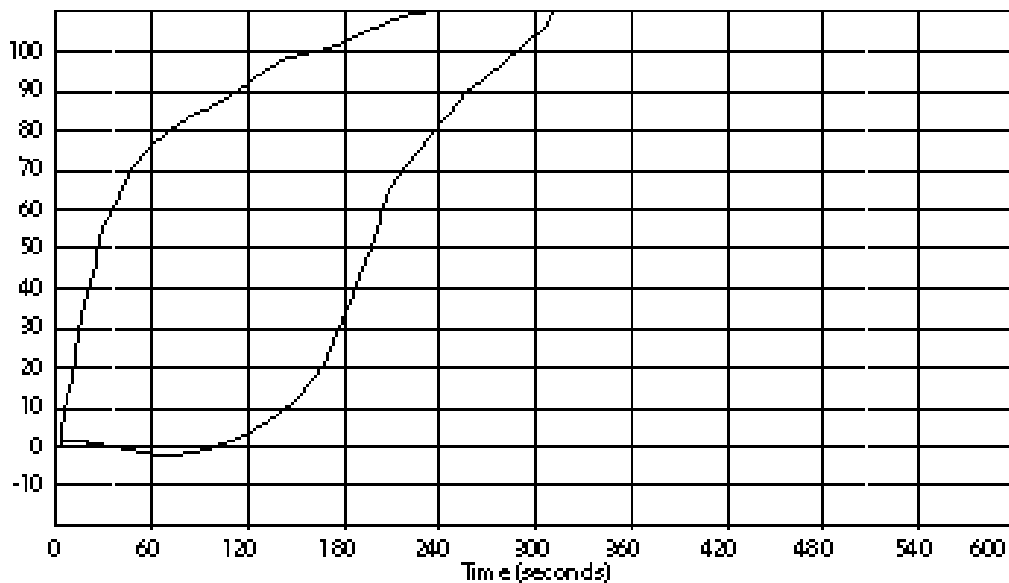

Fig. 2: Showing no inhibition of platelet aggregation with ADP and arachidonic acid

use of either low or high doses of aspirin in multiple large trials.[22]

2. Drug interaction: Most important is with ibuprofen. Ibuprofen is able to bind the COX-1 binding site of aspirin and via stearic hindrance may prevent aspirin from binding and exerting its antiplatelet effect.[23]

3. Cigarette smoking enhances platelet function.

4. Diabetes and hypercholesterolemia – by means of producing free radicals decreases response to antiplatelet therapy.

Biologic/ cellular factors[24]

1. Alternate pathways of platelet activation.

2. Failure to inhibit catecholamine-mediated platelet activation e.g. exercise, mental stress, epinephrine.

3. Overexpression of COX-2 mRNA. COX-2 is found in other tissues and in varying amount in platelets. It is inducible by cytokines. This induction of COX-2 provides an alternative pathway for prostaglandin-H2 production. The degree of expression of COX-2 in platelets is variable. People with aspirin resistance may aggregate their platelets through COX-2 mechanisms.

4. Regenerated COX-1 activity in macrophages and vascular endothelial cells

5. Erythrocyte-induced platelet activation

Table 2 : Long-term clinical studies of aspirin resistance

Reference (year)	Patients studied	Aspirin dose (mg/day)	Follow-up duration	End-point(s)	Results
Grotemeyer et al[7] (1993)	Stroke patients (n=180)	1500	24 months	Stroke/MI/ vascular events	10-fold lower risk in aspirin responders
Grundmann et al [9] (2003)	Thromboembolic diseases (CVD/PVD cerebrovascular) (*n*=53)	100	>60 months	Recurrent ischemic stroke/ TIA	34% of patients with recurrence due to irin nonresponder
Mueller et al [10] (1997)	Peripheral vascular disease (n=100)	100	18 months	Arterial reocclusion	87% higher risk in aspirin resistant patients
Gum et al [11] (2003)	Stable CVD (*n*=326)	325	679±185days	Death/MI/ CVA	24% *v.* 10% (aspirin resistant vs sensitive) status
Eikelboom et al [19] (2002)	Subgroup analysis of HOPE study (*n*=976)	75–325	5 years	MI/stroke/ CV death	1.8-times higher risk

CVD: cardiovascular disease; PVD: peripheral vascular disease; HOPE: Heart Outcomes Prevention Evaluation; PFA: platelet function assay; MI: myocardial infarction; CVA: cerebrovascular accident; TIA: transient ischemic attack

6. Generation of B2-isoPGF2 alpha binds to thromboxane receptors

Genetic[24]

1. Polymorphism of vWF receptor gene
2. Polymorphism collagen receptor
3. Functional single nucleotide polymorphism of COX-1 gene
4. Platelet glycoprotein IIIa polymorphism. The platelet glycoprotein IIb/IIIa complex is the receptor for fibrinogen and mediates platelet aggregation. Polymorphism exists in the IIIa subunit of this receptor with patients being PI A1/A1 homozygous, Pl A1/A2 heterozygous or Pl A2/A2 homozygous. Patients displaying either the Pl A1/A2 or Pl A2/A2 polymorphism have been shown to be less responsive to the antiplatelet effect of aspirin.[25] But, the main cause for aspirin resistance may be a yet undescribed genetic abnormality.

TYPES OF ASPIRIN RESISTANCE

Aspirin resistance was classified into three types by Weber et al.[26]

1. Aspirin resistance type I (pharmacokinetic type) - There is no effect on collagen-induced platelet aggregation or thromboxane formation while taking aspirin 100 mg/day for at least 5 days. While there is inhibition of platelet aggregation in vitro suggesting intra- and inter-individual variability in pharmacokinetics when aspirin is used at low doses.

2. Aspirin resistance type II (pharmacodynamic type). It is characterized by the inability of aspirin to inhibit platelet thromboxane formation both in vivo and in vitro.

3. Aspirin resistance type III (pseudo-resistance). It is characterized by inhibition of thromboxane formation in vivo but not in vitro. This type of aspirin resistance was designated 'pseudo-resistance', because, in these patients, aspirin exerted the expected pharmacodynamic effect, i.e., inhibition of platelet thromboxane formation.

CLINICAL SIGNIFICANCE OF ASPIRIN RESISTANCE

A few long-term follow-up clinical studies have suggested that aspirin resistance is indeed clinically important (Table 2).[7,9-11,19] Eikelboom et al[19] studied the relationship of aspirin resistance with increased risk of cardiovascular events. The study showed that there was a 3.5 times higher risk of cardiovascular death in aspirin resistant group. Grotemeyer et at[7] in a cohort of 180 patients with stroke, at a follow-up of 2 years, major clinical vascular events were seen in 40% vs. 4.4% of aspirin resistant as compared to aspirin responders. Similarly, in 100 patients undergoing peripheral balloon angioplasty, Mueller et al[16] reported an 87% higher risk of reocclusion on follow-up in patients who failed to show an appropriate response to aspirin. Grundmann et al[9] found that 34% of patients with recurrent cerebrovascular ischemic events were aspirin non-responders. Gum et al[11] noted an increased risk (24%) of composite end-points of death, MI or cerebrovascular accident (CVA) as compared to aspirin-responsive patients (10%) over a mean follow-up period of 679±185 days among 326 patients. Chen et al[12] reported that 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.

MANAGEMENT OF ASPIRIN RESISTANCE

There are no specific recommendations regarding the management of aspirin resistance.

1. General measures- Patient compliance

2. Increasing aspirin dose- Data from Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) and Blockage of the Glycoprotein IIb/IIIa Receptor to Avoid Vascular Occlusion (BRAVO) studies show that increasing aspirin dose is not useful.[7,28]

3. Combining with other antiplatelet agents-Clopidogrel inhibits platelet aggregation via ADP receptor and therefore may represent an important therapeutic alternative. Recent trial Clopidogrel versus Aspirin in Patients at Risk of

Ischemic Events (CAPRIE)) has shown a modest superiority of clopidogrel monotherapy over aspirin monotherapy.[29] Both CURE[27] and Clopidogrel for the Reduction of Events During Observation (CREDO) support the superiority of dual antiplatelet therapy over monotherapy. However resistance to other antiplatelet agents has also been reported. In a recent study up to 4.7% of the patients undergoing coronary stenting developed thrombotic stent occlusion despite intensive clopidogrel treatment.[30] An ongoing randomized study of clopidogrel for high risk atherothrombotic risk and ischemic stabilization management and avoidance (CHARISMA) trial – evaluating the combination of aspirin alone vs. aspirin + clopidogrel in both secondary prevention and high risk primary prevention will help in settling scores of these issues.[31]

CONCLUSION

Aspirin resistance has been estimated to exist, anywhere from 5% to 45% of the population. Considering the standard guidelines for the use of aspirin and our dependence on it to decrease adverse events by its antiplatelet action, this prevalence of aspirin resistance is very important and of great alarming clinical importance. Aspirin resistance generally describes the failure of aspirin to produce an expected biological response (i.e., inhibition of platelet aggregation) or to prevent atherothrombotic events. 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, uniformly accepted diagnostic criteria, the uncertain clinical relevance of aspirin resistance, and the absence of a proven therapeutic intervention for affected individuals.

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