REVIEW ARTICLE

Aspirin Resistance

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Summary:

Aspirin is the widely used cheap antiplatelet agent in coronary diseases and ischemic stroke patients. Unfortunately for the last two decades the term ''Aspirin resistance" (AR) has been evolved due to its' failure to protect the aspirin users against major cardiovascular events. This is a clinical syndrome which can be defined operationally as the failure of aspirin to inhibit platelet aggregation, platelet activation, or ThromboxaneA2 (TXA2) production. Although the nonmodifiable factors like the PlA1/A2 polymorphism in the GPIIIa platelet receptor have been identified as responsible for AR , bioavailability of aspirin affected by patient noncompliance, insufficient aspirin dosage and concomitant nonsteroidal anti-inflammatory drug (NSAID) use should be considered first as a responsible for this resistance. Optical platelet aggregometry or method of Born using platelet-rich plasma (PRP) traditionally is used to assay platelet activity. But 'VerifyNow' and 'IMPACT' two simple tests can be considered as point-of-care tests to assay AR. As there is no consensus about definition, no gold standard test to separate AR from treatment failure to aspirin , it is more appropriate to say "treatment failure" to aspirin therapy rather using the term AR.

(J Bangladesh Coll Phys Surg 2013; 31: 92-96)

Introduction:

Aspirin is the most commonly used antiplatelet drug and for more than 100 years it represents a cornerstone in the primary and secondary prevention of cardiovascular diseases most notably myocardial infarction and stroke. The US Preventive Services Task Force and the American Heart Association recommended aspirin use for all men and women whose 10-years risks are > 6% and 10% or more respectively. They also expect that in all these patient categories, including secondary prevention, acute MI and acute occlusive stroke, as well as primary prevention, increased and appropriate use of aspirin will prevent large numbers of premature deaths and myocardial infarctions (MIs).[1] But based upon various platelet function tests and the fact that patients experience vascular events despite taking aspirin, it is now established that a significant fraction of aspirin treated patients (upto 57%) is resistant to the antiplatelet effects of the drug.[2-4] Diabetic patients including a significant portion of metabolic syndrome patients have a high rate of chance to develope such a resistance and this is proved by a high rate of entry of new platelets into the circulation of diabetic patients.[5,6]

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Received: 7 February, 2013 **Accepted:** 18 March, 2013

Methodology:

Pub Med was searched in June 2012 for all English-language publications including the search terms 'Aspirin resistance', 'Definition and mechanism of aspirin resistance', 'Aspirin resistance or treatment failure' from 1990 onwards. The full articles for selected identified records that were thought to be potentially relevant were collected. The references in these articles were also checked for additional relevant information.

Definition of Aspirin Resistance (AR):

Although the term "aspirin resistance" was created almost two decades ago, it is still not defined. Even a working definition is not set. The term has been used to describe a number of phenomenon, including the inability of aspirin to: (a) protect individuals from thrombotic complications, (b) cause a prolongation of bleeding time, (c) inhibit thromboxane biosynthesis or,(d) produce an anticipated effect on one or more *in vitro* tests of platelet functions.[7] So the clinical syndrome of aspirin resistance can be defined operationally as the failure of aspirin to inhibit platelet aggregation, platelet activation, or TXA2 production.[7] There is no criteria for distinguishing true resistance from treatment failure, and there is no consensus on whether the definition of aspirin resistance should be based on clinical outcomes, laboratory evidence, or both.[2,7]

The mechanism/s for aspirin resistance: Although the exact mechanism of AR has not yet been fully

established, it is almost certainly multifactorial and due to a combination of clinical, biological, and genetic properties affecting platelet function. These can be subdivided into factors affecting bioavailability of aspirin, mechanisms intrinsic to platelet function, and extrinsic factors that affect platelet function. These are shown in table-I.

Table-I

Potential Mechanisms of Aspirin Resistance

Aspirin Bioavailability	
	Patient noncompliance
	Insufficient aspirin dose
	Concomitant nonsteroidal anti-inflammatory drug use
Intrinsic Platelet Function	
	Increased platelet turnover
	Platelet polymorphisms in-
	Cyclooxygenase-1
	Glycoprotein VI
	Glycoprotein Ia (á2-integrin)
	Glycoprotein IIIa (â3-integrin)
Extrinsic Factors Affecting Platelets	
	Remnant-like particles from platelets
	Polymorphism in factor XIII (Val34Leu)
	Induced cyclooxygenase-2 expression
	Prostaglandin H2 transfer to platelets
	RBC-derived substances
	Catecholamines
	C-reactive protein
	Tobacco use

Lack of patient compliance and aspirin dosage are two important factors responsible for AR. Another important factor affecting aspirin action is reduced bioavailability of this drug by concomitant use of NSAID.[8] NSAID, like aspirin, exhibit their anti-inflammatory effects through inhibition of enzyme cyclo-oxygenase (COX). NSAIDs compete with aspirin for the same binding site on COX. NSAID-induced COX inhibition is reversible. When NSAIDs are ingested before a patient takes aspirin, the aspirin (plasma half-life of 15 minutes) can be cleared from the blood before it irreversibly inhibits COX. If NSAID is given after aspirin ingestion, aspirin get times for it's irreversible binding to COX before competition with NSAID. Increased production of platelets associated with increased platelet turnover has been demonstrated in patients with immune thrombocytopenia and patients who have undergone peripheral blood stem cell transplantation. In these specific cases, newly formed platelets containing active COX-1, which is expressed at the earliest stages of megakaryocytopoiesis, might provide a pool of platelets with active COX-1 to offset the pool of older circulating platelets inhibited by COX-1. Also, newly formed platelets might contain small amounts of COX-2 that is not vulnerable to aspirin inhibition.[9,10] Therefore, any mechanism that can increase platelet turnover (eg, increased consumption) potentially might contribute to aspirin resistance. Genetic factors are also important, as these factors are nonmodifiable. At least 50 polymorphisms in 11 genes are found. The PlA1/A2 polymorphism in the GPIIIa platelet receptor is the most frequently observed polymorphism.[7] This polymorphism is associated with increased thrombin formation. A potential role of -765G/C polymorphism (rs20417) in COX-2 gene also has been found with AR in ischemic stroke patients.[11] The role of nucleated cells (endothelium, monocytes) and hyper-cholesterolemia are also to be claimed to develope AR.[11] Aspirin is thaught to be less effective in clinical conditions like diabetes mellitus, heart failure and in obese but exact mechanism is unknown. Statins use improve aspirin response. No association is found between aspirin response and age, body mass index, education, smoking status, family history of cardiovascular disease, and duration of aspirin use and not related to parameters such as HbA1c and low-density lipoprotein (LDL) or any dyslipidaemia, hypertension, and concurrent use of other medications such as beta blockers, angiotensin-converting enzyme inhibitors (ACEIs) and calcium channel blockers.[12] Factors extrinsic to platelets include the appearance of tissue derived COX-2, remnant-like particles (RLPs) that activate platelets, activation of platelets by RBCs, the presence of factor XIII polymorphisms, and activation of platelets by epinephrine, tobacco use, and C-reactive protein.[13]

Platelet function tests: Various platelet function tests have been developed and used to assess platelet function. Because aspirin inhibits platelet activation, these assays can be used to assess aspirin resistance. Table-II showing different Laboratory tests of aspirin resistance.

Table-II

Laboratory tests of aspirin 'resistance'

Basis of Test	Name of Test	Advantages	Disadvantages	Test reported to predict clinical aspirin 'resistance' (i.e. MACE)
In vivo cessation of blood flow by a platelet plug	Bleeding time	*In vivo* test Physiological	Insensitive High inter-operator coefficient of variation Can leave scar	No
In vitro cessation of high shear blood flow by a platelet plug	PFA -100®	Simple and rapid Low sample volume No sample preparation Whole blood assay High shear	Dependent on VWF and hematocrit No instrument adjustment	Yes
Shear-induced platelet adhesion	IMPACT® (cone and plate(let) analyzer)	Point-of-care Simple and rapid Low sample volume Whole blood assay High shear	Instrument not yet widely available.	No
	Aggregometry in response to AA and ADP (turbidometric)	Widely available	High sample volume Sample preparation Labor intensive	Yes
Platelet-to-platelet aggregation	Aggregometry in response to ADP and collagen (impedance)	Whole blood assay	High sample volume Timeco-nsuming	Yes
	VerifyNow® (Ultegra RPFA) with AA or propyl gallate cartridge	Point-of-care Simple and rapid Low sample volume No sample preparation Whole blood assay	No instrument adjustment	Yes
Activation-dependent changes in platelet surface	Platelet surface P-selectin, platelet surface activated GPIIb-IIIa, leukocyte-platelet aggregates in response to AA (flow cytometry)	Low sample volume Whole blood assay	Sample preparation Requires flow cytometer and experienced operator	No
	Serum thromboxane B_2	Directly dependent on aspirin's target: COX-1	Time consuming	No
Activation-dependent release from platelets	AA- or collagen-induced platelet thromboxane A_2 production, as measured by thromboxane B_2	Directly dependent on aspirin's target: COX-1	Sample preparation Time consuming	No
	Urinary 11-dehydro	Directly dependent on aspirin's target: COX-1	Dependent on renal function Potential	Yes

(AA, arachidonic acid; COX-1, cyclooxygenase 1; MACE, major adverse clinical events; PFA-100, platelet function analyzer-100; RPFA, rapid platelet function analyzer)

COX enzymes but results from this method do not correlate well with those of other platelet function tests.[21]

Conclusion:

Aspirin resistance can be a major health issue, potentially increasing the number of vascular events in patients on aspirin therapy. Patient's compliance, aspirin dosage, NSAID interaction, obesity, diabetes mellitus, infection or inflammation should keep in mind before thinking AR. Even though there are different laboratory tools that can be used to analyze the antiplatelet effects of aspirin, more work is needed to standardize and validate these tests. Most healthcare providers will address the issue by altering patients' current therapy although the treatment for aspirin resistance is still controversial and not standardized. As more research is being focused on this issue, testing for aspirin resistance can be used to help detect patients that may be at risk or have some degree of resistance and to possibly initiate alternative therapies.

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Optical platelet aggregometry or method of Born using PRP traditionally has been the test of choice for assessing platelet function.[14] These traditional studies are performed using PRP and platelet aggregation is determined by using optical platelet aggregometers. Aggregation is usually induced by adding a platelet agonist (i.e AA, collagen, epinephrine) to the PRP. The amount of platelet aggregation is related to the amount of light that passes through the solution, and the results are reported in units of percentage of light transmission on a scale of 0-100%. More recently various whole blood, point of care analyzers have become commercially available including the Platelet Function Analyzer 100 (PFA-100®), Impact®, and the VerifyNow® aspirin assay. These devices are less cumbersome and more convenient to use compared to the traditional tests using the optical method.[15] The PFA-100 analyzer functions by aspirating a blood sample through a capillary tube and through a small slit aperture cut into a membrane coated with collagen-epinephrine or collagen-ADP. The closure time in seconds for a platelet plug to occlude the slit aperture is inversely related to platelet activity. A value of 193 or less is considered normal, and values greater than 300 are considered non-closure. This analyzer is a convenient tool for measuring platelet aggregation, but its role in measuring aspirin resistance has not been appropriately evaluated. The VerifyNow Aspirin Assay detects platelet aggregation based on agglutination of platelets on fibrinogen-coated beads detected by an optical turbidimetry method. Results are expressed in aspirin response units with a value of 550 or more defined as aspirin resistance. Several other methods have been developed for use in prospective trials to quantify aspirin resistance. The TXA2 metabolite, 11-dehydrothromboxane B2 (11-dehydroTXB 2), can be measured in urine to estimate the activity of functioning COX enzymes. Although results from this method do not correlate well with those of other platelet function tests,[16] 11-dehydroTXB2 levels after aspirin therapy significantly correlate with an adverse prognosis in high-risk patients.

Discusion :

Aspirin-related compounds are among the oldest known medicinal substances, with stone tablets documenting the use of willow leaf (a source of salicylic acid) dating back to the Sumerian period.[17] Controversy surrounding the use of aspirin can be traced back to the Greek empire, when Hippocrates was a proponent of willow bark for pain, whereas Dioscorides preferred coriander.[18] We will never know whether Dioscorides was merely resistant to the beneficial properties of aspirin because thousands of years later we are still trying to understand and define the individual variability seen with its use. Although AR has been defined operationally as the failure of aspirin to inhibit platelet aggregation, platelet activation, or TXA2 production,[7] it is not accepted as a consensus view and also there is no consensus whether the definition of aspirin resistance should be based on clinical outcomes, laboratory evidence, or both. Moreover there is no criteria for distinguishing true resistance from treatment failure.[2] Reduced bioavailability is a parameter that theoretically is straightforward to rule out. Lack of patient compliance would reduce aspirin bioavailability, and this should always be considered. Another factor that affects aspirin bioavailability is proper aspirin dosage. In the 2002 Antithrombotic Trialists' Collaboration meta-analysis, 15 to 30 mg/d of aspirin was sufficient to inhibit COX-1 in most patients and was as effective as higher dosages. Efficacy data show that 81, 162, and 325 mg/d are reasonably equivalent, and 81 to 162 mg/d are used commonly for treatment. The optimal aspirin dosage for primary prevention of cardiovascular events is unknown. The US Preventive Services Task Force states that 75 mg/d is as effective as higher dosages, and the American Heart Association recommends a dosage range of 75 to 160 mg.[19,20] NSAID interfere aspirin action but this can be avoided by administration of NSAID after the patient has taken aspirin or not used at all by aspirin-treated patients. Practically speaking, if aspirin resistance is identified by one method or another, these simple bioavailability issues should be considered first, and, if present, they should be identified and corrected. Although historically, optical platelet aggregometry using PRP traditionally has been the test of choice for assessing platelet function, assay is cumbersome by requiring high volume sample preparation and also requires a specialized laboratory setting. Two point of care tests, 'VerfyNow' and 'IMPACT', use whole blood and no need for sample preparation. But instruments are not widely available. The TXA2 metabolite, 11-dehydroTXB 2 can be measured in urine to estimate the activity of functioning