# 23 Aspirin Resistance: Does it Exist?

**Abstract:** Aspirin irreversibly inhibits platelet cyclooxygenase-1 (COX-1). Aspirin resistance as defined by its inability to inhibit COX-1 enzymes in platelets, but such a situation does not exist. By using this definition aspirin resistance is very rare. A specific rapid laboratory test such as, either arachidonic acid (AA) induced platelet aggregation or AA induced malondialdehyde production in platelet rich plasma, is needed to test aspirin sensitivity to platelets. The reports on so-called aspirin resistance are usually due to non-compliance of aspirin intake or consumption of inadequate dose of aspirin. In addition, data generated from using non specific platelet function tests have also added confusion to this observed phenomenon of aspirin resistance.

## INTRODUCTION

Blood platelets play a very important role in the pathogenesis of heart attack and stroke.[1-4] Therefore, several million individuals all over the world take aspirin, an irreversible inhibitor of the cyclooxygenase enzymes, for the prevention of heart attack and stroke.

***Aspirin*** *as a therapeutic drug has been in use for over hundred years. Its use as an anti-platelet drug was recognized in the early 1970s. For the secondary prophylaxis of acute vascular events, it is the most useful and cost effective drug. Large number of clinical studies using aspirin has demonstrated that at any given risk, irrespective of the diseases state, aspirin at low to medium dose (80-160 mgs) is as effective as any other anti-platelet drug.*[5,6]

However in recent years, there is considerable concern regarding the development of aspirin resistance in certain group of patients suffering from heart disease. According to an article in the New York Times (Andrew Pollack 2004), anywhere from 5-40% of the individuals taking aspirin for secondary prophylaxis are non-responders.[7] Aspirin resistance is poorly understood and is ill-defined. However, if there is real lack of aspirin effect on platelet function in any patient, then it is important for him/her to know the existence of such a condition, so that alternate therapy can be recommended. According to some physicians non-compliance seems to be a significant mediator of poor out- come. Platelets exposed to aspirin may exhibit variable response to different agonists. Although this phenomenon is described as aspirin resistance, lack of an uniform definition or specific diagnostic method makes it difficult to detect this condition, under clinical situations. Aspirin resistance has been described in cardiovascular diseases, cerebrovascular diseases and peripheral arterial diseases. How much of this observed phenomenon is due to aspirin resistance is debatable. Because, many of these studies have used non-specific platelet function tests to determine aspirin resistance. In the absence of a specific reliable assay to monitor aspirin resistance, the results obtained from some of these studies are misleading and add to the confusion. There is a great need for the development of a simple, platelet specific, rapid, cost-effective assay for monitoring true aspirin resistance in high-risk individuals.

## Platelet Physiology

Blood platelets interact with a variety of soluble agonists such as epinephrine and adenosine diphosphate, many insoluble cell matrix components, including collagen, laminin, and biomaterials used for the construction of invasive medical devices.[8] These interactions stimulate specific receptors and glycoprotein-rich domains (integrins and non-integrins) on the plasma membrane of platelets and lead to the activation of intracellular effector enzymes (Fig. 1). The majority of the regulatory events appear to require free calcium. Ionized calcium is the primary bioregulator, and a variety of biochemical mechanisms modulate the level and availability of free cytosolic calcium. Major enzymes that regulate the free cytosolic calcium levels via second messengers include phospholipase C, phospholipase $A_2$, and phospholipase D, together with adenylyl and guanylyl cyclases. Activation of phospholipase C results in the hydrolysis of phosphatidyl inositol 4, 5-bisphosphate and formation of second messengers 1, 2-diacylglycerol and inositol 4, 5-bisphosphate ($IP_3$). Diglyceride induces activation of protein kinase C, whereas $IP_3$ mobilizes cytosolic calcium from internal membrane stores. Elevation of cytosolic calcium stimulates phospholipase $A_2$ and liberates arachidonic acid. Free arachidonic acid is transformed to a novel metabolite, thromboxane $A_2$ by fatty acid synthetase (COX-1, cyclooxygenase). Thromboxane $A_2$ is the major metabolite of this pathway and plays a critical role in platelet recruitment, granule mobilization and secretion. Secretory granules contain a variety of growth factors, mitogens and inflammatory mediators. Secretion of granules promotes p-selectin and CD40 expression on the platelet membrane. Furthermore, activation also promotes the expression of acidic lipids on the membrane and tissue factor expression, thus making these cells procoagulant. Fully activated platelets can modulate the function of other circulating blood cells such as leukocytes, monocytes, macrophages as well as vascular endothelial cells. Agonist-mediated stimulation of platelets promotes the expression of an epitope on glycoprotein 11b/111a receptors. Activation of this receptor is essential for the binding of circulating fibrinogen. Fibrinogen forms a bridge between individual platelets and facilitates the thrombus formation. Von Willebrand Factor (vWF) binds platelet GP1b1X complex only at high shear rate unlike fibrinogen, which can bind platelets at low shear. Up-regulation in signaling pathways will increase the risk for clinical complications associated with acute coronary events. Down-regulation of signal transduction mechanisms may precipitate bleeding diathesis or stroke.

## ARACHIDONIC ACID METABOLISM

Arachidonic acid is a 20 carbon polyunsaturated fatty acid ($20:4w^6$) found in membrane phospholipids. Cell activation stimulates phospholipase $A_2$, which facilitates the release of this fatty acid from phospholipids. AA is converted to prostaglandin (PG) endoperoxides ($PGG_2/PGH_2$) by cyclooxygenase (Prostaglandin G/H synthase; COX1) (Fig. 2). Cyclooxygenase exists in 2 iso-forms, COX-1 and COX-2. COX-1 is present in nearly all cells, while COX-2 is normally absent from cells but may be produced in response to inflammatory stimuli. These transient metabolites are converted by thromboxane synthetase to thromboxane $A_2$, which is the major metabolite of this pathway in platelets.[9] Whereas, in vascular tissues, the endoperoxides generated by COX-1 are transformed by prostacyclin synthetase to prostacyclin ($PGI_2$). Thromboxane is a potent platelet agonist and a vasoconstrictor. Prostacyclin is an anti-platelet compound and exerts vasodilatory effects on vascular tissues. Thus, from a single substrate (AA), two pharmacologically opposing vasoactive prostanoids are generated by platelets and vascular tissues. Aspirin selectively acetylates the hydroxyl groups of a single serine residue (position 529) in the prostaglandin G/H synthase and causes irreversible inhibition of the activity of this enzyme.[10-13] Inhibition of PG synthase results in the decreased conversion of AA to PG endoperoxides, $PGG_2/PGH_2$. Molecular mechanisms involved in aspirin-mediated inhibition of prostaglandin G/H synthase are well documented.[13] Aspirin works by inhibiting the

prostaglandin-producing enzyme cyclooxygenase, which converts arachidonic acid into prostaglandins. The anti-platelet action is through irreversible inhibition of platelet cyclo-oxygenases (COX-1) at serine residue 529.

The Thrombosis Research Laboratory at the University of Minnesota, under the supervision of Dr. Rao, has used platelet aggregometer for monitoring arachidonic acid induced activation of platelets, which is dependent upon the availability of PG endoperoxides and thromboxanes. Using this simple technique they have demonstrated that by and large, aspirin resistance is a rare phenomenon in normal healthy individuals. In their platelet function testing studies over three decades they have come across aspirin insensitivity in only one subject. In this subject, the platelets were found to be deficient in cyclo-oxygenase activity and as such failed to respond to arachidonate, as they were incapable of metabolizing AA to PG endoperoxides and thromboxane.[14] Researchers from the Artificial Heart Institute, Salt Lake City, published a report indicating that the bovine platelet cyclooxygenase is not inhibitable by oral dose of aspirin.[15] Rao, et al examined this phenomenon and found that if the aspirin was administered as a suppository through the anal route, the drug was quite effective inhibiting platelet cyclooxygenase.[16] In the earlier study since aspirin was administered orally it was probably metabolized in the stomach to salicylic acid before reaching the circulating blood. Salicylic acid is not an inhibitor of cyclo-oxygenase.

To demonstrate drug-induced refractoriness to aspirin, Rao et al used a short acting inhibitor, Ibuprofen.[17] This drug when used first antagonized the action of aspirin. In other words, in individuals administered with ibuprofen, aspirin did not have any effect on the platelet function. They hypothesized that ibuprofen was interfering with action of acetyl salicylic acid at the active site of the enzyme. When the active site is occupied by another drug, aspirin will not be able to interact with this site and acetylate the amino acid. Since ibuprofen is a short acting drug, platelets regain their response to arachidonate by next morning. If there were any effect of aspirin on these platelets, the platelets would fail to respond to arachidonate with aggregation even the next day. These studies clearly demonstrated that ibuprofen treated platelets exposed to aspirin responded to the action of arachidonic acid with irreversible aggregation upon recovery from the action of ibuprofen, suggesting that aspirin failed to inhibit COX-1 enzymes in the presence of ibuprofen.

Rao and co-workers at the University of Minnesota have discovered an intrinsic phenomenon called "membrane modulation" which is capable of securing irreversible aggregation of drug-induced refractory platelets; the novel mechanism is modulated by the stimulation of the alpha-adrenergic receptors and restores the sensitivity of drug-induced refractory platelets to the action of agonists.[18,19] This observation may partially explain why none of the currently available anti-platelet drugs are effective in the total prevention of *in vivo* platelet activation and formation of thrombi. Aspirin and Clopidogrel only inhibit one of the many platelet activation mechanisms. Therefore, they do not offer full protection from the risk of acute vascular events. Furthermore, their studies have demonstrated that when aspirin treated platelets are subjected to interact with exposed sub-endothelial cell matrix under flow conditions, there is more adhesion and aggregation of aspirin exposed cells than normal platelets. Though aspirin reduces the thrombus formation on the exposed cell matrix components, it does not prevent the formation of massive aggregates. Furthermore, when epinephrine was used in such flow conditions, adrenergic receptor modulation restored the ability for thrombus formation of aspirin exposed platelets.[19] It is essential to learn who is truly resistant to aspirin as defined by its inability to inhibit platelet COX-1, so that appropriate measurements or alternate prophylactic therapy could be administered. Even all those who are considered responders to the aspirin therapy may need additional protection, if their platelets are still sensitive or hypersensitive to other agonists such as collagen exposed on sub-endothelium after endothelial damage or plaque rupture, or have a hyper coagulation pathway. Incorrect definition of aspirin resistance based on the variation in incorrect detection methods used, and lack of data from large clinical trials has hampered the

advancement of knowledge in this area.[1-4] There is a great need for the development of point-of-care assay systems which are capable of detecting not only individuals who develop resistance to currently available anti-platelet drugs aspirin or clopidogrel, but also those who are hyper responsive to platelet activation by different agonists like collagen or sub-endothelium and those who have pro-thrombotic coagulation mechanisms.

## CONCLUSION

***Aspirin resistance in normal human population is a very rare phenomenon.*** Aspirin irreversibly inhibits platelet cyclooxygenase. Since these cells lack DNA they are unable to synthesize this enzyme like other blood cells. Half life of aspirin in circulating blood is fairly short (few minutes). Therefore, new platelets with fully active COX-1 enzymes are released into the blood stream constantly. In addition, circulating epinephrine or adenosine diphosphate can potentiate the action of other agonists and restore the function of aspirin exposed cells. The use of aspirin in secondary prophylaxis of vascular disease is cost effective. However, we should be concerned as to whether the low to medium dose of aspirin or that matter clopidogrel offers complete protection to all individuals at risk for acute vascular events. Methods currently used for monitoring aspirin resistance are non-specific. We need point-of-care assays to monitor not only anti-platelet therapies but also anticoagulant therapies, in order to customize the treatment of individuals at risk for acute vascular events.

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