Review Article

Aspirin Resistance: Clinical Significance

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Salicylates have been in use for the management of pain and inflammation for centuries. However, the stable form of this drug (acetyl salicylic acid), which was developed for therapeutic applications in the name of aspirin, has been in use for little over a century. Aspirin is the most cost-effective antiplatelet drug of choice for the management and secondary prevention of acute vascular events. Several hundred clinical studies have demonstrated the beneficial effect of aspirin in preventing or reducing the occurrence of acute vascular events. Aspirin at as little as 80–160 mg/d has been shown to offer significant benefits under a variety of platelet-related clinical complications. However, in recent years, there is a great concern that significant number of patients on aspirin prophylaxis may develop resistance to this therapy and thereby increase their chances of having acute vascular events. Several recent studies have demonstrated that aspirin resistance plays a critical role in the management of thrombotic conditions. Therefore, we feel that thorough understanding of this observed phenomenon is clinically important. In this article, we describe the platelet-related clinical complications leading to thrombotic conditions, try to clarify the confusion associated with the phenomenon of aspirin resistance, discuss methods available for determining "at risk" patients, and speculate on some alternate therapies.

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 (1–5). These interactions stimulate specific receptors and glycoprotein-rich domains (integrins and nonintegrins) on the plasma membrane of platelets and lead to the activation of intracellular effector enzymes. 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 A2, 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-diayclglycerol and inositol 4,5-bisphosphate (IP3). Diglyceride induces activation of protein kinase C, whereas IP3 mobilizes cytosolic calcium from internal membrane stores. Elevation of cytosolic calcium stimulates phospholipase A2 and liberates arachidonic acid. Free arachidonic acid is transformed to a novel metabolite, thromboxane A2 by fatty acid synthetase (COX-1, cyclooxygenase). Thromboxane A2 is the major metabolite of this pathway and plays a critical role in platelet recruitment, granule mobilization, and secretion (2–5). 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 pro-coagulant. Fully activated platelets can modulate the function of other circulating blood cells such as leukocytes, monocytes, macrophages, as well as vascular endothelial cells (ECs). 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

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Willebrand Factor (vWF) binds platelet GP1b1X complex only at high shear rate unlike fibrinogen, which can bind platelets at low shear. Upregulation in signaling pathways will increase the risk for clinical complications associated with acute coronary events. Downregulation of signal transduction mechanisms may precipitate bleeding diathesis or stroke.

Arachidonic Acid Metabolism

Arachidonic acid is a 20-carbon polyunsaturated fatty acid (20:4w6), found in platelet membrane phospholipids. Platelet activation stimulates phospholipase A2, which facilitates the release of this fatty acid from membrane phospholipids. AA is converted to prostaglandin (PG) endoperoxides (PGG2/PGH2) by cyclooxygenase (prostaglandin G/H synthase; COX1)). These metabolites are converted by thromboxane synthetase to thromboxane A2, which is the major metabolite of this pathway in platelets (2,3). While, in vascular tissues, the endoperoxides generated by COX1 are transformed by prostacyclin synthetase to prostacyclin (PGI2). Thromboxane is a potent platelet agonist and a vasoconstrictor. Prostacyclin is an antiplatelet 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 PG G/H synthase and causes irreversible inhibition of the activity of this enzyme. Inhibition of PG synthase results in the decreased conversion of AA to PG endoperoxides, PGG2/PGH2. Molecular mechanisms involved in aspirin-mediated inhibition of PG G/H synthase are well documented.

Screening and Diagnosis

Activation of Circulating Blood Cells and Inflammation

There is increasing evidence suggesting that the inflammatory mediators play a pathogenic role in the atherogenesis as well as in acute coronary syndromes (6–10). Several recent studies suggest that inflammatory cytokines such as tumor necrosis factor (TNFa), interleukin (IL-1), and various chemokines (IL-8) may activate matrix metalloproteinases and induce degradation of connective tissue and promote apoptosis of cells in the atherosclerotic lesion, thus promoting plaques destabilization and rupture. There is considerable evidence to show that platelets contribute significantly to the pathogenesis of acute coronary syndromes by facilitating thrombus formation. They may also trigger acute coronary events by other mechanisms including stimulation of an inflammatory response within the atherosclerotic plaque. They secrete a wide range of growth factors and inflammatory mediators. In addition, they activate other cells such as monocytes and macrophages and promote the expression of tissue necrosis factor and tissue factor. They also modulate the function of leucocytes and promote the expression of chemotactic and adhesive properties of ECs as well as IL-1 production in smooth muscle cells. Increased leucocyte–platelet aggregation has been demonstrated in acute myocardial infarction (MI) and cerebral infarction. It has been shown that release of CC-chemokines RANTES by platelets triggers the arrest of monocytes on inflamed atherosclerotic endothelium. Furthermore, in vulnerable plaque significant amounts of tissue factor expression also has been demonstrated. It is clear from the available evidence that several platelet-derived factors, both membrane bound (p-selectin, p- CD40L) as wells soluble (s-selectin, sCD40L), are involved in the inflammatory response mediated by the activation of platelets, leucocytes, monocytes, macrophages, and ECs. Some studies have demonstrated the presence of platelet activation markers such as p-selectin and CD40 in patients with MI. There is some evidence to suggest that over-expression of CD40L on platelets is correlated with the need for reangioplasty. Expression of this protein promotes the expression of adhesion molecules, chemokines and inflammatory cytokines, and recruitment of leucocyte within the vulnerable plaque. Levels of circulating markers of inflammation, C-reactive protein (CRP) has been found to be higher in patients with unstable coronary syndromes. Furthermore, according to Harvard researchers, persistent elevation of CRP in patients with unstable angina seems to be a good predictor of future coronary events such as ischemia and MI (11).

Vascular Dysfunction

Functional and structural changes in the arterial wall precede the development of atherosclerosis, obstructive coronary artery disease (CAD), and may even serve as an early marker for the hypertensive disease (1–5). Function and structural changes of vascular ECs are modulated by a variety of thrombogenic factors as well as antithrombogenic factors (12–16). Some of the vasoactive

compounds released by the ECs include vasodilatory compounds such as adenosine, prostacyclin, and nitric oxide and vasoconstrictory molecules like cyclo-oxygeanse-derived endothelium-dependent constriction factor (EDCF), hypoxia-induced EDCF and endothelin. Lipid peroxides, oxidized lipids and lipoproteins promote the formation of vasoconstrictors by platelets. These lipid mediators inhibit enzymes that promote the formation of vasodilators by the healthy endothelium and lower endogenous production of vasodilators. Alterations in the balance between platelet-associated vasoconstrictors and EC-derived vasodilators result in the vascular dysfunction (15). This is probably the earliest stage at which one can detect the manifestation of the arterial dysfunctions, hypertension, and atherosclerosis. Indeed, one can classify the risk according to the level of EC dysfunction and additional CAD risk factors present. One can use acetylcholine, L-arginine, and nitric oxide synthetase inhibitor, LNNMA and monitor the flow response in the forearm, to determine the degree of EC dysfunction (16). Alternatively, one can use CV Profilor (DO-2020, Hypertension Diagnostics, Eagan, Minnesota) or Periscope (Genesis Medical Systems, Hyderabad) and monitor the pulse waveform of the small arteries (17,18).

Risk Profiling for Acute Cardiovascular Events

Framingham study initiated some 50 years ago established for the first time significant risk associated with the elevated levels of blood cholesterol to the development of CAD (19–24). Since that time many studies have demonstrated the increased risk for CAD with the increase in total blood cholesterol. In view of these findings over the years many lipid-lowering drugs have been developed and tested. National Cholesterol Education Program has been developed by the National Institutes of Health, USA, and appropriate guidelines have been established for the better management of risks associated with abnormal lipid metabolism. Indeed Framingham risk assessment protocol as well as PROCOM risk assessment calculator uses the common risk factors for CAD for assessing the risk for coronary events. Although lipid abnormalities have been shown to increase the risk for CAD, their exact role in precipitating acute coronary events has not been demonstrated. Therefore, it is not clear as to whether or not lipid-lowering drugs offer full protection against CVD events.

Results of one of the clinical trials with high-risk patients provide partial answer to this question. Postmenopausal women have increased risk for developing heart disease and often results are fatal. Several studies are in progress to evaluate the beneficial effects or otherwise of hormone replacement therapy (HRT). Outcome of one of the multicenter placebo control study (Heart and Estrogen/Progestin Replacement Therapy, HERS) sheds some light on the importance of hematological parameters in the precipitation of acute coronary events (25). HERS study was a randomized trial of estrogen and progestin for the secondary prevention of CAD in postmenopausal women (2700, 4.1 years). Antihypertensive agents and lipid-lowering drugs were used as concomitant therapy. HRT significantly lowered serum lipids and associated CAD risk promoters. However, HRT did not lower the cardiovascular events associated with postmenopausal conditions, No studies were done on the hemostatic variables in this clinical study. The result of this study clearly suggests the importance of hemostatic variables in precipitating coronary events. In a randomized placebo-controlled 12-week study it was found that there was a shift in the pro-coagulant anticoagulant balance toward a pro-coagulant state following HRT (26). Short-term HRT also has been shown to increase circulating activated platelets. There is considerable evidence to suggest a role for activated platelets in mediating inflammatory response. Such inflammatory response can lead to the elevation of CRP in the circulation (26). These observations suggest that just the management of the known classical risk promoters for CAD may not be sufficient to protect the postmenopausal women from the risk for future coronary events. It is important to look at the role of platelets, coagulation factors and degree of inflammation in precipitating coronary events in these high-risk individuals.

Studies on the Use of Aspirin as an Inhibitor of Cyclooxygenase Enzymes

Salicylates have been in use for the management of pain and inflammation for centuries. However, the stable form of this drug (acetyl salicylic acid), which was developed for therapeutic applications in the name of aspirin, has been in use for little over a century. Aspirin is the most cost-effective antiplatelet drug of choice for the management and secondary prevention of acute vascular events. Several hundred clinical studies have demonstrated the beneficial effect of aspirin in preventing or reducing the occurrence of acute vascular events (28–32). Aspirin at as little as 80–160 mg/d has been shown to offer significant benefits under a variety

of platelet-related clinical complications. Single oral doses of 10–100 mg of aspirin can significantly inhibit the platelet PG synthase activity (28). The inhibitory effect of aspirin on circulating platelets in the blood is for a very limited time and probably occurs in the portal circulation. The half-life of aspirin is very short (15–20 min), but sufficient to inhibit PG synthase of circulating platelets. Since these cells lack DNA and the ability to resynthesize the enzyme, the dysfunction caused by aspirin cannot be overcome. Therefore, platelets exposed to aspirin lose the ability to make the prostanoids completely. However, one should keep in mind that once the aspirin is hydrolyzed to salicylic acid, ability to inhibit PG synthase is lost. Hence the platelets produced from the marrow after the aspirin is hydrolyzed will have active PG synthase. Approximately 10% of fresh platelets are added on to the circulating blood every day. Although aspirin-treated blood platelets do not make PGs, they respond with aggregation to the stimulation by PG endoperoxides and thromboxane. Fresh platelets formed after the hydrolysis of aspirin can synthesize prostanoids and these newly formed metabolites of AA can cause aggregation of aspirin-exposed platelets. In view of the fact that aspirin irreversibly inhibits PG synthase, it is possible to take advantage of repeated low-dose aspirin to achieve a cumulative effect (28–45). Even doses as low as 30–50 mg aspirin taken daily will suppress platelet thromboxane synthesis significantly in 5–10 days. Vascular tissues on the other hand have the ability to resynthesize PG G/H synthase (40). Therefore, these cells can recover the enzyme activity following aspirin exposure. It is. therefore, possible to develop a strategy to promote the biochemical selectivity of aspirin in terms of inhibition of platelet PG synthase. This is done by modification of the drug delivery, so the amount of drug delivered is just enough to inhibit platelet enzymes in the peripheral circulation and spare the systemic effect on vascular endothelium (40, 45).

As mentioned earlier, aspirin is metabolized rapidly and the major metabolite, salicylic acid is a poor inhibitor of platelet PG synthase. Therefore, it is essential to develop appropriate strategies to maximize the beneficial effect of this novel drug. A dose as low as 20 mg taken daily reduces the platelet thromboxane formation by more than 90%. However, it is generally believed that higher doses are essential for preventing thromboxane-dependent platelet activation. Studies by Wilson et al. demonstrated that maximal plasma concentration of 12 umol/L could be achieved by a single oral 50 mg dose of enteric-coated aspirin (43). This dose was found sufficient to cause significant inhibition of platelet function and daily ingestion of low-dose aspirin demonstrated a cumulative effect. In a separate study, McLeod et al. used doses ranging 50–3900 mg of aspirin and monitored platelet function, bleeding time, and concluded that maximum dysfunction was obtained with daily doses of about 100 mg and no further changes were observed in these studies with higher doses (44). Several workers have demonstrated the efficacy of low-dose oral aspirin in preventing platelet thromboxane production (28–30, 44). Indeed one of these studies has demonstrated beneficial effect of a dermal aspirin preparation on selective inhibition of platelet PG synthase, sparing the prostacyclin biosynthesis (64). It is very well established that 100 mg of aspirin per day is sufficient to significantly reduce the platelet thromboxane production (28–30, 46, 47, 66–68). Furthermore, studies by McLeod et al. have shown that dosages higher than 100 mg/d do not produce any greater inhibition of platelet function or enhance bleeding times (44). Therefore, it is reasonable to conclude that 80–160 mg aspirin per day should be the choice for an ideal preventive protocol (67). However, there is considerable room for improvement to maximize the benefits by better understanding the pharmacology of aspirin and platelet physiology (28–30). It is possible to customize the aspirin treatment based on the individual patient needs. One can monitor the platelet PG synthase activity following aspirin ingestion and recommend a dose that is appropriate (60, 67). It is possible to monitor the platelet response to agonists such as ADP or arachidonate and determine the degree of inhibition by aspirin-like compounds (44). In order to get maximum inhibition of platelet COX-1 enzymes, continuous release aspirin formulations can be developed and tested against currently available aspirin formulations. Platelets are produced and released constantly to the circulation. Therefore, a time-release aspirin, which would make available small amounts of aspirin into the circulation, may be effective. For instance, a 100 mg formulation capable of releasing 10 mg acetyl salicylic acid per hour may be better than a preparation which releases all of its active principle in a short span of time. Using the strategy of slowing down the release of active principle, newer formulations could be used effectively to provide needed amounts of the drug into circulating blood at regular intervals. These novel formulations may also provide selectivity of aspirin action by preventing platelet thromboxane production and sparing the endothelial prostacylcin synthesis. McLeod et al. studied the effect of various

doses of aspirin (50, 100, 325, 1000 mg) on platelet and vascular tissues (69). They did not observe inhibition of urinary 6-keto-PGF1 alpha production at low doses of 50 and 100 mg. They attributed these findings to the differential and selective inhibition of platelet function and the sparring effect of vascular COX1 enzymes. Sullivan and associates studied the effect of two different doses of aspirin on platelet function and TXA2 production (71). Platelet function in healthy volunteers was inhibited by both the doses (75 and 300 mg). Low dose failed to inhibit completely TXB2 production 24 hours later, whereas 300 mg aspirin did. Even alternate day regimen of these doses prevented platelet function and significantly inhibited the urinary levels of the 11-keto-TXB2. In a separate study, in healthy volunteers, formation of thrombin (fibrinopeptide A; FPA), alpha granule release (betathromboglobulin; beta TG), and thromboxane (TXB2) were monitored in vivo, in blood emerging from a template bleeding incision (72). At the site of plug formation significant platelet activation and thrombin generation was observed as indicated by 110-, 50-, and 30-fold increase in FPA, beta TG, and TXB2, within the first minute. A low-dose regimen (0.42 m/kg/d for 7 days) caused greater than 90% inhibition of TXB2 formation in both bleeding time and clotted blood in these studies, suggesting critical role of platelet activation at the site of hemostatic plug formation. In a study to evaluate the effect of low-dose aspirin (0.5 and 15 mg/kg/d) on platelet and renal prostanoids, Wilson et al. monitored serum TXB2 and urinary 6-keto PGF1 alpha (73). Serum TXB2 level was reduced to 3% of control by low dose and to 0.1% by the higher dose. Urinary TXB2 was reduced only to 68% by low-dose aspirin and to 51% by high dose. Urinary 6-keto-PGF1 alpha was not reduced by either dose. Based on their observation, they concluded that low-dose aspirin could significantly affect platelet PG production without affecting stimulated release of PGI2 production.

Clinical Studies on the Use of Aspirin

The two major clinical trials on aspirin concluded that ingestion of 160 mg per day or 325 mg alternative day provided significant benefit in preventing fatal events associated with CAD (46, 47). While, a 10-year trial involving nearly 40,000 women aged 45 and older with no evidence of cardiovascular disease found that a regular alternate day low-dose (100 mg) aspirin was effective in reducing the incidence of stroke, but it did not have any effect on the incidence of heart attacks (74). They concluded that the reasons for any sex-based differences in the efficacy of aspirin for primary prevention are unclear. According to Minnesota Heart Survey, about 6% of healthy women under age 65 and 30% of those over 65 take low-dose aspirin to prevent acute vascular events. Data from this primary prevention study do not apply to women who already have had a heart attack or heart surgery or diagnosed with CAD. For such women, as found in men, regular daily low dose (80–160 mg) of aspirin clearly reduces the risk of developing acute coronary events.

Several earlier studies evaluated the effect of aspirin on normal healthy volunteers as well as patients with various vascular diseases (71–136). However, earlier studies did not report prevalence of any aspirin resistance. Zucker et al. evaluated the effect of low-dose aspirin (0.45 mg/kg/d) and a high dose (900 mg/d) in type 11 hyperlipoproteinemic subjects (75). They found that low-dose aspirin effectively inhibited platelet function in these patients. Increased platelet thromboxane production has been described in several disorders including type-2 diabetes and type 11a hypercholesterolemia. This increased production of TXB2 in hypercholesterolemic patients is attributed to abnormal cholesterol levels in these patients. It has been shown that even a low dose of aspirin (50 mg/7 days) significantly reduces 11-dehydro-TXB2 in these patients (76). The effect of low-dose aspirin has been evaluated in patients with diabetes, coronary heart disease, MI, cerebrovascular disease, peripheral artery disease, and a variety of surgical procedures (71–84). Diminno et al. studied the effect of single doses of 100 and 1000 mg aspirin for 1 month in normal volunteers and patients with diabetic angiopathy (77). They found that a dose schedule of aspirin, which may suffice in normal volunteers, was not effective in patients with diabetic angiopathy. Contrary to this observation, Terres et al. found that a low dose of aspirin (100 mg) caused significant inhibition of platelet function in both healthy subjects and patients with coronary heart disease (78). Similarly, a low dose (0.45 mg/kg/d) was found adequate for selective inhibition of TXA2-related platelet function in patients recovering from MI (79). Looks like the results on the effect of low-dose aspirin vary considerably, depending upon the type and stage of disease, dose of aspirin, and severity of procedure. In a study evaluating the effect of low-dose aspirin (100 mg) on hematological activity of left ventricular (LV) thrombus in anterior wall acute MI (AMI), Kupper et al. found that low dose had no effect on the incidence

of hematologic activity and embolic potential of LV thrombosis in anterior wall AMI (80). On the other hand, a low-dose aspirin (40 mg/d) taken daily was found to be as effective as higher doses in preventing platelet functional responses in patients who had recent cerebral ischemia (81). Uchiyama et al. evaluated the effect of low-dose aspirin, ticlopidine, and a combination of both these drugs in patients with cerebral ischemia (82). Aspirin alone markedly inhibited platelet aggregation induced by AA, partially inhibited aggregation induced by ADP and did not inhibit aggregation by platelet activating factor. Combination of these drugs inhibited aggregation by all agonists. Rao et al. demonstrated that in healthy volunteers, low doses of aspirin (40–80 mg) had no inhibitory effect on the response of platelets to ADP, epinephrine and thrombin, but effectively inhibited the platelet response to threshold concentrations of AA (29, 30). Epinephrine at concentrations too low to cause aggregation restored the sensitivity of aspirin-treated platelets to AA (84–91). This phenomenon, in which weak agonists restore the sensitivity of drug-induced refractory platelets to the action of other agonists, was described from our laboratory as "mechanism of membrane modulation" (84–91).

Aspirin Resistance

Studies from our laboratory for the first time demonstrated that one could induce drug-mediated resistance in platelets to the action of aspirin (92). In this study, the subjects were given a short-acting inhibitor of COX1, ibuprofen. This was followed by administration of a full-strength (325 mg) aspirin. Ibuprofen-mediated inhibition of COX1 enzyme lasts for a short time, whereas aspirin-induced inhibition is irreversible. Ibuprofen-treated platelets recovered their sensitivity to the action of AA by 24 hours. While aspirin-treated platelets failed to respond to the action of AA even after 24 hours. In those subjects who had ingested aspirin after taking ibuprofen first, aspirin failed to inhibit irreversibly the COX1, suggesting that ibuprofen molecules effectively prevented the acetylation of COX1enzyme by aspirin. One of the earliest work describing "nonresponders" and "responders" evaluated the effect of low-dose aspirin and a thromboxane synthetase inhibitor dazoxiben (UK3724B) in healthy subjects (83). These studies demonstrated that low-dose aspirin and ingestion of two dazoxiben tablets prevented the release of granules from platelets in response to AA in some individuals (responders) and not in others (nonresponders). These subtle differences in response of platelets to various drugs as well as differences in response to various agonists may be critical when considering the outcome of acute vascular events. For instance, collagen seems to exert its effect by multiple mechanisms. In a study using aspirin monoclonal antibodies to 11b-111a receptor and fibrinogen, it was demonstrated that there exist at least three mechanisms by which collagen activates platelets: (a) GP11b-111a associated activation, (b) PG-dependent pathway, (c) alternate pathway responsible for 20–30% platelet aggregation (95).

Several recent studies have demonstrated drug resistance in patients with a variety of vascular diseases (101–132). This subject currently is a very hot topic and has made national headlines. Andrew Pollack published an article in July 2004 in New York Times on this subject titled "For Some, Aspirin May Not Help Hearts" (95). According to this article, 5–40% of aspirin users are "nonresponders" or "resistant" to the drug. In the same article, he cites the opinion of Dr. Daniel I. Simon, the associate director of interventional cardiology at Brigham and Women's Hospital, Boston, which reads as follows: "They are taking it for stroke and heart attack prevention and it's not going to work". He also reports the opinion of Dr. Michael J. Domanski, head of clinical trials unit at the NIH. In his opinion, the nonresponders may represent a huge number of patients. According to Dr. Deepak L. Bhatt, director of interventional cardiology, Cleveland Clinic, aspirin resistance is associated with worst outcome. Professor Eric Topol, Chairman, Cardiovascular Medicine Cleveland Clinic, USA, states "Aspirin resistance carries high risk, with over 20 million Americans taking aspirin to prevent heart attacks or strokes, it is important that further work to be done to confirm our findings and develop a rapid detection method. He also assures that for individuals with aspirin resistance, there are excellent alternatives."

These observances from healthcare providers and researchers raise number of issues. Do we know enough about aspirin resistance? What is the prevalence of aspirin resistance in healthy population? What causes this resistance to develop in patient populations? Are there specific, rapid, cost-effective tests available? What alternative long-term treatments are available if patients are resistant to common antiplatelet drugs such as aspirin and clopidogrel? Should the doses of these drugs used for therapy be increased? Should we drop the use of these drugs in nonresponders? These observations also raise the need to develop newer and effective antiplatelet drugs. We need to find answers to these and other

emerging questions soon. In the next few paragraphs a brief overview of what is known about the prevalence of aspirin resistance, clinical findings, and methodologies available will be provided.

The first and foremost need at this time is to standardize a definition of aspirin resistance. The mechanism of action of aspirin is very well documented (33–39). The drug acetylates the platelet COX1 enzyme and irreversibly inhibits its ability to convert AA to PG endoperoxides (36, 39). In the absence of COX-1 enzyme activity, platelets do not respond to AA stimulation with aggregation. Weak agonists such as ADP or epinephrine depend on the formation of PG endoperoxides to initiate secondary wave of aggregation and promote release of platelet granule contents (29). Therefore, weak agonists fail to induce platelet aggregation and release granules from aspirin-treated platelets. Failure of AA, ADP, and epinephrine to cause aggregation of platelets more or less establishes drug-induced platelet dysfunction. If platelets obtained from individuals who have ingested a full strength aspirin respond with aggregation to the action AA, ADP, and EPI, and release their granule contents, then one can safely conclude that these platelets are resistant to aspirin action. Further proof for aspirin resistance of platelets can be provided by studying AA metabolism by such platelets, monitoring serum TXB2 levels, or urinary levels of TXB2 or its metabolite, 11-dehydro-TXB2. Methods are available to monitor all these parameters. According to Cattaneo, "aspirin resistant" should be considered as description for those individuals whom aspirin fails to inhibit thromboxane A2 production irrespective of the results of unspecific tests of platelet function (124).

Prevalence of Aspirin Resistance

SAspirin resistance has been poorly defined, variety of nonspecific methods have been employed to monitor the "aspirin resistance," and conflicting reports have been published on the rates of prevalence and outcome of continuing this therapeutic modality (95–111). Aspirin resistance has been reported in patients with cardiovascular, cerebrovascular, and peripheral vascular disease (95–111). Because of the differences in methodologies used to monitor this phenomenon and lack of a specific assay to determine the true aspirin resistance, there is considerable confusion and the true significance of this observation remains obscure (96–98). It also raises the question as to how we missed this phenomenon of drug resistance all these years. Large numbers of clinical trials have demonstrated the beneficial effects of aspirin therapy irrespective of the disease state (66). Is it possible that these earlier trials missed aspirin nonresponders? On the other hand, it is quite possible that only responders to the action of aspirin got the benefit of this therapy.

Studies in our laboratory over three decades have failed to show any aspirin resistance in normal healthy subjects. The only subject whose platelets failed to aggregate in response to AA stimulation was found to be deficient in platelet COX-1enzyme activity (86). Platelets obtained from this subject responded with aggregation when stirred with epinephrine and arachidonate, suggesting that PG endoperoxides and TAX2 are not essential to cause irreversible aggregation of platelets. There is not much data on the prevalence of aspirin resistance in general healthy subjects. In patients with various vascular diseases, the rate of nonresponders reported varies between less than 2% to over 60%. Since the methods used to monitor aspirin resistance in these reports are not specific, the prevalence rate published is debatable (95–111).

Hurlen et al. used the method of Wu and Hoak to determine the platelet aggregation ratio as a marker for assessing platelet function and evaluated the effect of aspirin (160 mg/d) in 143 patients who had survived MI (99–102). Based on their definition of nonresponders to the action of aspirin, they could only identify two subjects as primary nonresponders. Gum et al. from Cleveland Clinic studied 326 stable cardiovascular subjects on aspirin (325 mg/d) and tested aspirin sensitivity by platelet response to aggregating agents such as ADP and AA. They found 5.5% as nonresponders to aspirin and 24% as semiresponders (101). Gum and associates used the PFA-100, a method that measures platelet function, to determine aspirin resistance in their patient population (102)]. Based on the results of their studies with this methodology, they found 9.5% to be nonresponders to aspirin action.

Some studies have reported as high as 30–40% nonresponders of stroke or vascular disease patients and predicted >80% increase risk for a repeat event during a 2-year follow-up period (103–106). Eikelboom et al. analyzed baseline urinary levels of TXB2 metabolites 11-dehydro thromboxane B2 in 5529 patients enrolled in the Heart Outcomes Prevention Evaluation (HOPE) study (107). Of these subjects 488 were on aspirin regimen. On the basis of their findings they concluded

that in aspirin-treated patients, increased levels of urinary metabolite of TXB2 predict future risk of MI or cardiovascular death. The patients with the highest levels of urinary TXB2 metabolite had 3–5-fold higher risk of cardiovascular death compared to those in the lowest quartile. Another study reporting clinical outcomes of aspirin resistance is from Austria (104,106,109). In this study patients undergoing arterial angioplasty were on 100 mg aspirin per day. Platelet function was assessed by whole blood aggregometry. This study demonstrated that reocculsion at the sites of angioplasty occurred only in men for whom platelet dysfunction was evident by aggregometry (106). Zimmerman et al. identified aspirin nonresponders as those who had >90% inhibition of TXB2 formation in presence of 100 umol/L aspirin and 1 mmol/L arachidonate (110). In patients who had undergone coronary bypass surgery (CABG), AA and collagen stimulated formation of TXB2 was same before and after CABG, indicating that oral aspirin did not significantly inhibit platelet COX1. However, the in vitro studies with 100 umol/L aspirin on blood obtained from these subjects showed decreased TXB2 (>10%) in most samples studied. They concluded that platelet COX1 inhibition by aspirin is compromised for several days after CABG, probably due to an impaired interaction between aspirin and platelet COX1. This observation indicates how complex the issues are when evaluating the effect of antiplatelet drugs during and after interventional procedures. Sane et al. evaluated the effect of aspirin (325 mg/d/month) in patients suffering from congestive heart failure (111). These researchers used whole blood aggregometry (Chronolog Corp, PA, USA), platelet receptor expression by flow cytometry and PFA-100. Patients were considered nonresponders when four of the five parameters assayed were observed. Using this complex rating, persistent platelet activation was observed in 50 of the 88 patients (56.8%). These observations remind us of the inadequacy of the existing methods to detect what truly represents "aspirin resistance."

In our earlier articles (84–91) we described how epinephrine-mediated membrane modulation restores the response of COX 1 deficient platelets as well as those of aspirin-exposed platelets to the action of agonists such as AA, ADP and thrombin independent of bioactive meatabolites of AA. In our earlier studies, we also demonstrated that small quantities of endoperoxides or thromboxane generated from platelets or from some other source also could cause aggregation of aspirin-exposed platelets. Half-life of aspirin in circulation is relatively short and once the liver metabolizes it, the circulating salicylic acid has no inhibitory effect on platelet function. In addition, the bone marrow continuously produces fresh platelets and releases them into the blood. These newly released platelets contribute significantly to the circulating in vivo thromboxane.

Our own recent observations in India as well as several other earlier reports suggest that one of the better ways to monitor "at risk" patients is by monitoring the urinary metabolites of thromboxane and not on the basis of results of in vitro platelet function tests (101–107). Several recent studies have demonstrated that in spite of the inhibition of platelet COX enzymes, significant number of patients on aspirin prophylaxis had increased levels of urinary metabolites of thromboxane (107). In the Heart Outcomes Prevention Evaluation study in which over 5500 patients were enrolled, it was found that in aspirin-treated subjects, increased levels of urinary metabolite TXB predict future risk of MI and cardiovascular death (107). The patients with highest level of urinary thromboxane levels had 3–5-fold higher risk of cardiovascular death than those in the lowest quartile.

At the time of this writing it is not clear as to the exact source of in vivo thromboxane in patients undergoing aspirin prophylaxis. Clinical manifestation of aspirin resistance could be defined as occurrence of acute events such as MI, stroke, or PAD in patients in spite of aspirin prophylaxis and on the other hand the laboratory observations are based on altered platelet response to various agonists. The observed excess of in vivo thromboxane may be due to insufficient dose of aspirin in these subjects or because of lack of compliance or due to excess production of new platelets from the bone marrow or due to altered or accelerated aspirin metabolism by these individuals. However, what is evident from recent studies is that no matter what the source of this thromboxane, it puts the patients at risk for developing acute vascular events (101–107). As mentioned earlier, the levels of circulating levels of thromboxane and prostacyclin modulate the normal hemostasis. In view of this, it is better to monitor the levels of urinary metabolites as biomarkers for both these vasoactive molecules. This need has been well demonstrated in the studies on Non Steroidal anti-inflammatory drugs (NSAIDs), where the investigators tested the hypotheses that adverse cardiovascular events reported among Anti-inflammatory Prevention Trial

(ADAPT) participants were associated with increased ratio of urinary 11-dehydrothromboxane (TXB-M) to 2'3-donor-6-keto-PGF1 (PGI-M) attributable to NSAID treatments (135). Results of these studies showed that adverse cardiovascular events were significantly associated with higher urinary TXB-M/PGI-M ratio, which seemed to derive mainly from lowered PGIM.

In conclusion, platelet activation as well as the activation of coagulation cascade is modulated by a variety of mechanisms. Therefore, there is a great need to develop assays, which monitor global hemostasis (combined activation of platelet and coagulation pathways). Till we have such a point-of-care method available (PlaCor: Platelet Reaction Time Monitor [PlaCor Inc., Minneapolis, Minnesota], Aggredyne Platelet Function Monitor [Aggredyne, Houston, Texas]), it is better to use urinary metabolites of TXB/PGI as biomarkers to monitor "at risk" patients (136). Currently, well-documented and standardized methods are available to monitor patients at risk for developing acute vascular events. These methods involve measuring urinary metabolites of thromboxane or prostacyclin. Altered levels of the ratio between these metabolites or increased levels of urinary metabolites of thromboxane predict risk for future acute vascular events in patients, who are on aspirin prophylaxis. In addition, further studies are needed to develop newer and effective alternate antiplatelet therapies.

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