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Management of type-2 diabetes with anti-platelet therapies: special reference to aspirin

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1. ABSTRACT

Adult onset diabetes currently affects 380 million individuals worldwide and is expected to affect 380 million by 2025. Major defects contributing to this complex disease are insulin resistance and beta cell dysfunction. More than 80% of patients professing to type-2 diabetes are insulin resistant. Recent studies have shown that the Indian subcontinent ranks very high in the occurrence of Diabetes and Coronary artery disease (1, 2, 3). Patients with Type 2 diabetes carry an equivalent cardiovascular risk to that of a non-diabetic individual who has already experienced a coronary event. The risk of coronary artery disease in any given population seems to be 2-3 times higher in diabetics than non-diabetics. Inflammation, platelet activation, endothelial dysfunction and coagulation are the four processes, whose interplay determines the development of cardiovascular disease. In this article, we provide a brief overview on platelet physiology, vascular dysfunction, platelet hyper-function, and the role of platelet related clinical complications in diabetes mellitus and what is know about the management of this complex disease with anti-platelet drugs such as aspirin and Clopidogrel.

2. INTRODUCTION

Once considered a rich persons disease, type-2 diabetes has now become a high profile health concern in developing countries as well as in industrial nations (3). The number of people with diabetes is estimated to double worldwide in 20 years. Projected estimates (in million) for the top five countries include, India 32, China 21, USA 18, Indonesia 8.4, Japan 6.8 in 2000 and India 79.4, China 42.3, USA 30.3, Indonesia 21.3 and Japan 8.9 for 2010 (4). Diabetes is a serious, complex common metabolic disease. Management of this disease is quite costly in terms of health care burden, but is controllable or manageable. In the industrial nations this disease is associated with obesity and sedentary life style. Central abdominal adiposity by and large, predicts the development of type-2 diabetes, cardiovascular morbidity, and mortality in South Asians. Recent studies have demonstrated that South Asians have a very high incidence of type-2 diabetes as well as coronary artery disease (CAD). Elevated risk factors classically associated with CAD, do not adequately explain the increase risk for diabetes and CAD in patients with Syndrome X in Asians. Central abdominal obesity seems to be an independent risk factor for CAD in this population.

According to extensive studies done by epidemiologists in Chennai, India, body composition and fat distribution seems to be significantly different between Caucasians and Asian Indians (5-7). Type-2 diabetes accounts for 90-95 percent of all diagnosed cases of diabetes in adults. Usually it begins as an insulin resistance or a glucose intolerant disorder, in which the cells do not use insulin properly and as need for insulin raises the pancreas loses its ability to produce sufficient quantities of insulin. According to the landmark studies of Diabetes Control and Complications Trial (DCCT), after 30 years of diabetes, fewer than 1 percent of those receiving intensive glucose control in the DCCT had significantly impaired vision, kidney failure, or needed limb amputations (8). Currently sufficient knowledge exists on the pathogenesis and clinical complications of this disease and there are effective treatment options to manage this complex disorder and to reduce the long-term complications associated with this disease (8-12). In this overview we discuss some relevant aspects of diabetes management, as they relate to cardiovascular complications and what is known about anti-platelet therapy.

3. 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 (13-22) These interactions stimulate specific receptors and glycoprotein-rich domains (integrins and non-integrins) on the plasma membrane and lead to the activation of intracellular effector enzymes (23,24). The majority of the regulatory events appear to require free calcium (25, 26). Ionized calcium is the primary bio-regulator, and a variety of biochemical mechanisms modulate the level and availability of free cytosolic calcium (27, 28). Major enzymes that regulate the free 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 calcium from internal membrane stores (23-28). 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 (28-31). Secretory granules contain a variety of growth factors, mitogens and inflammatory mediators. Secretion of granules promotes p-selectin 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 (14-17) 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 signaling pathways may precipitate bleeding diathesis or hemorrhagic stroke (25, 26).

4. ARACHIDONIC ACID METABOLISM

Arachidonic acid (AA) is a 20 carbon polyunsaturated fatty acid (20:4w6) found in membrane phospholipids (31). 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; COX-1)). These transient metabolites are converted by thromboxane synthetase to thromboxane A_2, which is the major metabolite of this pathway in platelets (31-36). 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 (34). Thus from a single substrate (AA), two pharmacologically opposing vasoactive prostanoids are generated by platelets and vascular tissues (34-36). 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 (33). 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 (31-36).

5. PLATELET HYPERFUNCTION

Contribution of circulating blood platelets to the pathogenesis of atherosclerosis, progression of vascular complications related to diabetes is well documented (17-21). Patients with type-2 diabetes seem to have 2-4 fold increase risk of morbidity and mortality due to coronary artery disease (22, 39-42,). Platelets obtained from diabetic subjects show increased adhesiveness, hyperfunction both spontaneous as well as in response to agonists. These observed hyperfunctions are attributed to increase expression, activation or abundance of surface membrane receptors for agonists as well as cell matrix components; increased binding of fibrinogen, altered membrane fluidity, changes in activation mechanisms and signaling pathways. Several earlier studies (both in animal models and human subjects) have demonstrated platelet hyperfunction associated with hyperglycemia (43-48). In early 60s Bridges *et al* showed both *in vitro* as well as *in vivo*

administration of glucose increased platelet stickiness (41). Studies by Kwaan *et al* demonstrated that plasma from diabetic patients can enhance the response of normal platelets to the action of agonists (42).

With the discovery of prostanoids (prostaglandin endoperoxides, thromboxane and prostacylcin), there was considerable interest in showing a definite role for these lipid mediators in the diabetes related clinical complications (43). In early 80s, we explored the mechanisms involved in platelet hyperfunction in a rat model (43). In this novel study, drug-induced diabetic condition increased the ability of platelets to produce significantly higher levels of thromboxane upon stimulation. Whereas, stimulation of vascular tissues obtained from diabetic animals generated much lower quantities of prostacyclin compared to the tissues from control animals. We speculated that such an imbalance in the production of vasoactive metabolites will create a prothrombotic condition in diabetic subjects. However, in what turned out to be a novel experiment pancreatic islet cell transplantation restored the normal functional response of both the platelets and vascular tissues (43).

Mandal *et al* using whole blood aggregometry showed that platelet hperaggregation was present even at the time of diagnosis in patients with diabetes mellitus, even in the absence of any vascular complications (44). Various platelet activation markers such as CD62, CD63, PAC-1, annexin V and platelet derived microparticles (PDMP), have been used to follow the state of platelet activation in diabetic subjects. Some studies have suggested that PDMP may contribute to the development or progression of atherosclerosis (45). Whereas, a study from Germany demonstrated that in diabetics increased number of large platelets circulate in an activated state mostly in patients with angiopathy (46). Based on the results of their studies, they suggested three different mechanisms by which activated platelets modulate the prethrombotic state in the diabetic subjects: 1) Microembolization of the capillaries, 2) local progression of preexisting vascular lesions by secretion of constrictive mitogenic and oxidative substances; 3) trigger of the arterial thrombotic event (47). Therefore, there is reason to believe that activated platelets as well as PDMP may contribute significantly to the prethrombotic state in diabetes mellitus. Fattah *et al* evaluated haemostatic parameters such as prothrombin time, partial thromboplastin time, thrombin time, coagulation factor 11, V11, 1X, & plasma fibrinogen, ADP–induced platelet aggregation, protein C, alpha2-antiplasmin, PAI -1, and FDPs. They found high levels of fibrinogen, alpha2-antiplasmin, PAI-1 and lower levels of protein C. They concluded that altered state of these haemostatic parameters could lead to a prethrombotic tendency in diabetic patients (48). Studies by Deepa and Mohan demonstrated significantly elevated PAI-1 and tPA antigen levels in subjects with CAD and not so significant elevation in diabetic subjects (49)

Although several studies have suggested that hypersensitivity of platelets as well as hyper function of coagulation cascade predispose diabetic subjects to a prothrombotic state, there is not much of direct evidence to suggest that such conditions induce acute vascular events (46). Studies by Kawamori *et al* demonstrated a relationship between progression of atherosclerosis and *in vivo* platelet activation. They concluded from their studies that the progress of atherosclerosis in diabetes mellitus is associated with *in vivo* platelet activation and platelet activation does not occur in diabetics without carotid atherosclerosis (51). Nakajima *et al* using The Thrombotic Status Analyzer (TSA) found that elderly male subjects had prothrombotic state not because of platelet hyperaggregability but because of suppressed thrombolytic activity (52). Carr *et al* showed that both platelet function and clot structure may be altered in diabetes. They observed increased platelet contractile force as well as clot elastic modulus in diabetic patients with chest pain (53). Hyperglycemic spikes seem to play a role in vascular complications. Gresele *et al* demonstrated that shear stress-induced platelet activation, P-selectin and LIMP expression on platelets in the bleeding time blood, and urinary 11-dehydro-TxB2 excretion were increased after hyperglycemic clamping and not after euglycemic clamping (54).

Diabetes mellitus is associated with increased risk for atherosclerosis, thrombosis and stroke. Several earlier studies have demonstrated a critical role for platelets in diabetes-related clinical complications (44-54). Platelets of patients with diabetes have shown to be sticky, hypersensitive to the action of agonists, possess increased number of surface membrane receptors for binding fibrinogen as well as cell matrix components, enhanced arachidonate pathway, and increased phosphoinositol turnover leading to enhanced production of second messengers, and calcium mobilization and protein phosphorylation. Furthermore, activated coagulation pathway also has been demonstrated in the diabetic subjects. Further studies are needed to evaluate the specific role of platelet hyperfunction and hyper coagulation in the pathogenesis of atherosclerosis, thrombosis and stroke.

6. VASCULAR DYSFUNCTION

Functional and structural changes in the arterial wall precede the development of atherosclerosis, obstructive coronary artery disease and may even serve as an early marker for the hypertensive disease (55, 56). Function and structural changes of vascular endothelial cells (ECs) are modulated by a variety of thrombogenic factors as well as anti-thrombogenic factors (56). Some of the vasoactive compounds released by the ECs include vasodilatory compounds such as adenosine, prostacyclin and nitric oxide and vasoconstrictory molecules like, cyclooxygenase derived endothelium dependent constriction factor (EDCF), hypoxic-induced endothelium dependent constriction factor and endothelin. Lipid peroxides, oxidized lipids and lipoproteins promote the formation of vasoconstrictors by platelets (57). 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 results in the vascular dysfunction (58-60). 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 to determine the degree of EC dysfunction (61-64). Alternatively, one can use CV Profiler (DO-2020, Hypertension Diagnostics, Eagan, Minnesota) or the Periscope (Genesis Medical Pvt Ltd, Hyderabad, India) and monitor the pulse waveform of the small arteries (64, 65). Several earlier studies have shown that there is alteration in the pulse flow of blood in the diabetic subjects indicating the pathogenesis of vascular dysfunctions and arterial stiffness. Studies by Rema *et al* as part of their CURE project, have demonstrated that diabetic retinopathy is associated with Intima-Media thickness and arterial stiffness, suggesting these vascular alterations might predispose diabetics to micro-and macro angiopathy (66).

Several earlier studies have demonstrated that hyperglycemia, oxidative stress, platelet activation might predispose diabetic subjects to vascular dysfunction, atherosclerosis and acute vascular events (67 39-42,). In an earlier study, we demonstrated that drug induced hyperglycemia, results in the altered production of platelet metabolites of arachidonic acid and that of vascular tissues (43,). Similar alterations have been observed in the production of nitric oxide (NO) and endothelin-1. Ouvina *et al* have demonstrated increased levels of endothelin, von Willebrand factor, and soluble selectin in the plasma of diabetic subjects. (68). The authors concluded that presence of increased endothelial damage markers and low nitric oxide bioavailability in diabetic subjects could lead to a higher risk of development of thrombotic events. To demonstrate a similar alteration in the vasodilators and vasoconstrictors, Tretjakovs *et al* studied AA incorporation into phospholipids and nitric oxide end products in diabetic subjects (69). Their study demonstrated that development of diabetes in CAD patients decreased the ability to produce platelet derived NO and enhanced AA incorporation into phospholipids. Whereas, Huszka *et al* demonstrated in their study that endothelial derived-NO is reduced in diabetes suggesting vascular damage (70). They concluded that decreased NO production my lead to increased platelet activation in these individuals.

Alterations in the balance between various bioactive metabolites generated by platelets and vessel wall leads to either hyper- or hypo- function of platelets. Level of cytosolic free calcium and degree of actin assembly and phosphorylation of contractile proteins dictates the degree of platelet activation (56, 57, 25-28). Pellegatta *et al* studied both resting calcium levels and agonist mediated rise in the levels and concluded that poor metabolic control (HbA1c.8.0) altered calcium homeostasis as well as stimulation-response coupling (71). Schaeffer *et al* based on their results concluded that platelets of diabetic subjects have enhanced calcium signaling due to excessive superoxide production (72). Fukuda *et al* studied phosphorylation of myosin light chain (MLC) in platelets of diabetic subjects as well as age and sex matched controls (73). They found that basal MLC phosphorylation was significantly higher in platelets of diabetic subjects irrespective of their age. Results of these studies and the earlier observations demonstrate that hyperglycemia, oxidative stress, platelet activation and vascular dysfunction contribute significantly to the pathogenesis of diabetes mellitus, a chronic metabolic disorder.

7. CLINICAL USE OF ASPIRIN

Aspirin (acetyl salicylic acid) is the most cost-effective anti-platelet drug available for primary and secondary prophylaxis of acute coronary syndromes. Aspirin at a dose of 80-160 mgs has been shown to offer significant benefit in alleviating platelet-related clinical complications in a variety of thrombotic situations (74-93). Single oral doses of 10-100 mgs of aspirin can significantly inhibit the platelet PG synthase activity (74). The inhibitory effect is fast and probably occurs in the portal circulation. The half-life of aspirin is very short (15-20 minutes) 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 loose 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 prostaglandin synthase is lost. Hence the platelets produced from the marrow after the aspirin is hydrolyzed, will have active prostaglandin synthase. Approximately 10% of fresh platelets are added on to the circulating blood every day. Although aspirin treated blood do not make prostaglandins, they respond with aggregation to the stimulation by prostaglandin 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 prostaglandin synthase, it is possible to take advantage of daily low-dose aspirin to achieve a cumulative effect. Even doses as low as 30-50 mg aspirin taken daily will suppress platelet thromboxane synthesis significantly in 5 to 10 days.

Vascular tissues on the other hand, have the ability to resynthesize prostaglandin G/H synthase (74). 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 prostaglandin 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 (75, 76). Several studies have demonstrated the feasibility of this approach and various control release or timed release formulations have been developed for this novel therapy (75-77).

As mentioned earlier, aspirin is metabolized rapidly and the major metabolite, salicylic acid is a poor inhibitor of platelet prostaglandin synthase. Therefore, it is essential to develop appropriate strategies to maximize the beneficial effect of this novel drug. As low dose as 20 mg taken daily, reduces the platelet thromboxane formation by more than 90 percent. 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 12umol/L could be achieved by a single oral 50 mg dose of enteric-coated aspirin (84). 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 from 50-3900 mg of aspirin and monitored platelet function, bleeding time and concluded maximum dysfunction was obtained with daily doses of about 100 mg and no further changes were observed in these studies with higher doses (18). Several workers have demonstrated the efficacy of low-dose oral aspirin in preventing platelet thromboxane production (2-4, 18, 82). Indeed one of these studies has demonstrated beneficial effect of a dermal aspirin preparation on selective inhibition of platelet prostaglandin synthase, sparing the prostacyclin biosynthesis (76). Studies done with low-dose aspirin and the data generated by the two major clinical studies support the use of 80-160 mg aspirin per day as a prophylactic drug for the secondary prevention of clinical complications associated with platelet hyperfunction (19, 20).

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 (20, 21). It is very well established that 100 mg of aspirin per day is sufficient to significantly reduce the platelet thromboxane production (2-4, 20, 21, 78-80). Furthermore, studies by McLeod *et al* have shown that dosages higher than 100 mg per day do not produce any greater inhibition of platelet function or enhance bleeding times (81). Therefore, it is reasonable to conclude that 80-160 mgs aspirin per day should be the choice for an ideal preventive protocol (79). However, there is considerable room for improvement to maximize the benefits by better understanding the pharmacology of aspirin and platelet physiology (2-4). It is possible to customize the aspirin treatment based on the individual patient needs. One can monitor the platelet prostaglandin synthase activity following aspirin ingestion and recommend a dose that is appropriate (34, 79). 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 (18). In order to get maximum inhibition of platelet 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 (81). 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 COX enzymes. Sullivan and associates studied the effect of two different doses of aspirin on platelet function and TXA_2 production (83). Platelet function in healthy volunteers was inhibited by both the doses (75 and 300 mg). Low dose failed to inhibit completely TXB_2 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 (beta-thromboglobulin; beta TG), and thromboxane (TXB2) were monitored *in vivo*, in blood emerging from a template bleeding incision (85). At the site of plug formation significant platelet activation and thrombin generation was observed as indicated by 110 fold, 50 fold, and 30 fold increase in FPA, beta TG, and TXB2, within the first minute. A low dose regimen (0.42m/kg/day 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 haemostatic plug formation. In a study to evaluate the effect of low dose aspirin (0.5and 15 mg/kg/day) on platelet and renal prostanoids, Wilson *et al* monitored serum TXB2 and urinary 6-keto PGF1 alpha (85). Serum TXB2 level was reduced to 3% of control by low dose and to 0.1% by the higher dose. Urinary TXB_2 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 low dose aspirin could significantly affect platelet PG production without affecting stimulated release of PGI2 production.

Several earlier studies evaluated the effect of low dose aspirin on normal healthy volunteers as well as patients with various vascular diseases. However, earlier studies did not report prevalence of any aspirin resistance. Zucker *et al* evaluated the effect of low dose aspirin (0.45mg/kg/day) and a high dose (900mg/day) in type 11 hyper-lipoproteinemic subjects (86). 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 TXB_2 in hyper-cholesterolemic patients is attributed to abnormal cholesterol levels in these patients. Even a low dose such as 50 mg for 7 days significantly reduced 11-dehydro-TXB_2, in these patients (87). The effect of low dose aspirin has been evaluated in patients with diabetes, coronary heart disease, myocardial infarction

(MI), cerebrovascular disease, peripheral artery disease and a variety of surgical procedures. 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 (88). They found 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 a low dose of aspirin (100mg) caused significant inhibition of platelet function in both healthy subjects and patients with coronary heart disease (89). Similarly, a low dose (0.45mg/kg/day) was found adequate for selective inhibition of TXA2-related platelet function, in patients recovering from MI (90). Looks like the results vary considerably, depending upon the type and stage of disease, dose of aspirin, and severity of interventional procedures. In a study evaluating the effect of low dose aspirin (100mg) 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 (91). On the other hand, a low dose aspirin (40mg/day) daily was found to be as effective as higher doses in preventing platelet responses in patients who had recent cerebral ischemia (92). Uchiyama *et al* evaluated the effect of low dose aspirin, ticlopidine, and a combination of both these drugs in patients with cerebral ischemia (93, 94). 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, in healthy volunteers, low doses of aspirin (40-80mg) had no inhibitory effect on the response of platelets to ADP, epinephrine and thrombin, but effectively inhibited the platelet response to threshold concentrations of arachidonic acid. Epinephrine at concentrations too low to cause aggregation restored the sensitivity of aspirin-treated platelets to AA. 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" (95-103).

8. 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 (103). In this study, the subjects were given a short acting inhibitor of COX-1, Ibuprofen. This was followed by administration of a full strength (325mg) aspirin. Ibuprofen-mediated inhibition of COX-1 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 hrs. Whereas, aspirin treated platelets failed to respond to the action of AA even after 24 hrs. In those subjects who had ingested aspirin after taking Ibuprofen first, aspirin failed to inhibit irreversibly the COX-1 activity, suggesting Ibuprofen molecules effectively prevented the acetylation of COX-1 enzyme. One of the earliest work describing "non-responders" and "responders" to the action aspirin, evaluated the effect of low dose aspirin and a thromboxane synthetase inhibitor dazoxiben (UK3724B) in healthy subjects (94). 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 (non-responders). 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 exists at least three mechanisms by which collagen activates platelets; 1) GP11b-111a associated activation, 2) prostaglandin dependent pathway, 3) alternate pathway responsible for 20-30% platelet aggregation (104).

9. PREVALENCE OF ASPIRIN RESISTANCE

Aspirin resistance has been poorly defined, variety of non-specific 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 (105-121). Aspirin resistance has been reported in patients with cardiovascular, cerebrovascular, and peripheral vascular disease (107). 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 (107,108). It also raises the question as to how we missed this phenomenon all these years. Large numbers of clinical trials have demonstrated the beneficial effects of aspirin therapy irrespective of the disease state (78). Is it possible that these earlier trials missed non-responders? Is it possible that only responders 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 arachidonate was found to be deficient in platelet COX-1 activity (97). Platelets obtained from this subject responded with aggregation when stirred with epinephrine and arachidonate, suggesting PG endoperoxides and TAX_2 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 non-responders 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.

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 (160mg/day) in 143 patients, who had survived myocardial infarction (109, 110). Based on their definition, they could only identify two subjects as primary non-responders. Gum *et al* from Cleveland Clinic studied 326 stable cardiovascular subjects on aspirin (325mg/day) and

tested aspirin sensitivity by platelet response to aggregating agents such as ADP and AA. They found 5.5% as non-responders to aspirin and 24% as semi-responders (111). Gum and associates used the PFA 100, a method that is supposed to measure platelet function, to determine aspirin resistance in their patient population (112). Based on their studies with this methodology they found 9.5% to be non-responders to aspirin action.

Some studies have reported as high as 30-40% non-responders in stroke or vascular disease patients and predicted >80% increase risk for a repeat event during a 2-year follow up period (113-116). Eikelboom *et al* analyzed base line urinary levels of TXB2 metabolites 11-dehydro thromboxane B2 in 5529 patients enrolled in the Heart Outcomes Prevention Evaluation (HOPE) Study (117). 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 TXB_2 predict future risk of myocardial infarction or cardiovascular death. The patients with the highest levels of TXB_2 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 (113,118,119). 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 (113). Zimmerman *et al* identified aspirin non-responders as those who had >90% inhibition of TXB_2 formation in presence of 100umol/L aspirin and 1mmol/L arachidonate (83). In patients who had undergone coronary bypass surgery (CABG), AA and Collagen stimulated formation of TXB_2 was same before and after CABG, indicating oral aspirin did not significantly inhibit platelet COX-1 enzymes. However, the *in vitro* studies with 100umol/L aspirin on blood obtained from these subjects showed decreased TXB_2 (>10%) in most samples studied. They concluded that platelet inhibition by aspirin is compromised for several days after CABG, probably due to an impaired interaction between aspirin and platelet COX-1 enzymes. This observation indicates how complex the issues are when evaluating the effect of anti-platelet drugs during and after interventional procedures. Sane *et al* evaluated the effect of aspirin (325 mg/day/month) in patients suffering from congestive heart failure (left ventricular ejection fraction<40%) (121). They used whole blood aggregometry (Chronolog, Chronolog Corp, PA., USA), Platelet receptor expression by flow cytometry and PFA 100. Patients were considered non-responders when 4 of the 5 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".

10. MANAGEMENT OF DIABETES WITH ANTI-PLATELET THERAPY

The total number of people with type-2 diabetes worldwide was estimated to be 171 million in the year 2000 and it has been projected to increase to 366 million by 2030. The prevalence of diabetes seems to be higher in men than women, although there are more women with diabetes than men. The number of Americans with diagnosed diabetes is projected to increase by 165% from 11 million to 29 million by 2050. On the other hand, this kind of increase in the prevalence of diabetes, in India and China has been projected to take place in less than a decade. Researchers estimate that there are as many individuals with undiagnosed pre-diabetic conditions worldwide. The type-2 diabetes comprises 90% of the people with diabetes worldwide, and is by and large due to excess body weight and physical inactivity. In the year 2005 more than a million people died from diabetes related complications. World Health Organization projects, that death due to diabetes-related complications will increase more than 50% in the next 10 years. Both macrovascular and microvascular disease contribute significantly to the morbidity and mortality associate with this disease (122). Macrovascular disease, which affects the large vessels, such as coronary or lower extremities, lead to myocardial infarction, stroke or peripheral vascular disease. Greater than 80% of deaths in type-2 diabetes, are due to cardiovascular disease and stroke. Microvascular disease affects small vessels supplying blood to retina, nerves and kidneys. Microvascular dysfunction results in end organ damage leading to retinopathy, neuropathy or nephropathy. Since the emphasis in this overview is on anti-platelet therapy, we will briefly discuss clinical complications associated with platelet hyper-function and some of the known management strategies.

Several studies over four decades, have demonstrated hyperfunction of platelets of patients with diabetes (123-126). Animal studies as well as human studies have demonstrated increased production of thromboxane synthesis leading to hyper responsive platelets (43, 127). Some studies have demonstrated presence of younger platelets in circulation with more sensitive response to agonists (128-131). These experimental evidence as well as observed benefits from anti-platelet trials prompted various health care professionals and health care providers to suggest initiation of anti-platelet therapy for diabetes (78). American diabetes association has a position statement on "Aspirin Therapy in Diabetes" (132). According to the ADA recommendations, low dose aspirin therapy should be prescribed as a secondary prevention strategy. They also suggest low dose aspirin therapy for primary prevention in men and women with diabetes, who are at high risk for cardiovascular events (133). In UK, the guidelines and recommendation for diabetic patients includes, treatment with aspirin (75mg per day) or clopidogrel (75 mg per day), to be given continuously for long-term use. For primary prevention aspirin (75 mg) should be considered for all diabetic patients with well-controlled hypertension (134).

Anti-platelet drugs are used for both the primary and secondary prevention of cardiovascular complications in the diabetic subjects, although current guidelines are not consistent in their recommendations. By and large, there are three classes of anti-platelet drugs including, cyclooxygenase-1 (COX-1) inhibitors (aspirin), ADP

receptor (P2Y12) antagonists (thienopyridines) and platelet glycoprotein (GP) 11b/111a antagonists (134). It is very well established that people with diabetes have a 2 to 4 fold higher risk of dying from cardiovascular disease. People with diabetes have a complex pro-coagulant state, which also contributes to the increased risk of athero-thrombotic events (135,136). Anti-platelet therapy is a simple affordable intervention that can reduce the risk of acute vascular events significantly in this high-risk population. NHANES 111 data shows that 27% of people with diabetes are eligible for secondary prevention strategies, while additional 71% had at least one additional risk factor for atherosclerotic disease. Therefore, basically all individuals with diabetes are candidates for anti-platelet therapy, yet only 13% of the eligible patients are currently taking aspirin (137, 138). Miller and associates came to a similar conclusion in their study of a randomized trail of a decision supporting system. They concluded that despite clinical practice guidelines recommending anti-platelet therapy for patients with diabetes, there are still patients not receiving this beneficial therapy, particularly patients under 65, women and patients with without known CVD risks (138). They also recommended that effective methods to increase anti-platelet use should be considered at the national, community, practice and provider level. If this is the state of affairs in USA, it is hard to visualize the use of anti-platelet therapy for this population in developing and resource poor countries.

11. LIMITATIONS OF CURRENT STRATEGIES AND FUTURE DIRECTIONS

Aspirin is the most cost-effective drug of choice for the secondary prevention of acute vascular events worldwide (78). Aspirin acetylates COX-1 enzyme and prevents the formation of active metabolites of arachidonic acid by platelets. This inhibitory effect is irreversible, as platelets cannot resynthesize this enzyme. In addition to secondary prevention it can also be used for the primary prevention of thrombotic episodes. The American Diabetes Association (ADA) recommends the use of low-dose aspirin (80-160mgs) daily as a primary prevention strategy (132). Several clinical studies have demonstrated the beneficial effect of aspirin in diabetic patients and by and large, most of them have demonstrated significant benefit from the use of aspirin in these patients (139-42). However, results of these trials have been subjected to some criticism as they did not have large enough subjects and moreover not specifically designed for evaluating in diabetic population. The Japanese Primary Prevention study (JPAD) evaluated the use of low dose aspirin (81/100mg) in diabetic subjects (n=2540) for over 4 years. The incidence of fatal coronary events in this group was lower by 90% although there seems to be no significant difference in the non-fatal cardiovascular and cerebro-vascular events (141). ADA recommends use of low dose aspirin for the secondary prevention of acute vascular events in all diabetic patients. A meta analysis of 287 secondary prevention trials involving over 212,000 high risk subjects demonstrated that anti-platelet therapy reduced the incidence of acute vascular events by 23%. Furthermore, low dose aspirin was as effective as higher doses of aspirin (78). There is considerable debate about the aspirin resistance. However, observed discrepancy could be due to several other factors unrelated to the ability or inability of the aspirin to prevent COX-1 activity in the platelets of this population. It is well known that platelets of patients with diabetes have increased response to other stimulants. These subjects also seem to have considerable endothelial dysfunction. Therefore, those who manage these patients should evaluate both endothelial and platelet function in these subjects and develop appropriate customized anti-platelet therapy.

Clopidogrel seems to be the alternative choice or even the primary choice in some situations. CAPRIE trials examined the Clopidogrel (75 mg) versus Aspirin (325mg) in at risk patients (n=19185). Bhatt and associates analyzed the results of CAPRIE study with special reference to diabetic subjects (20% of the total subjects studied). According to their findings the reduction in the vascular events in the primary end points with Clopidogrel was not statistically different compared to aspirin in the non-diabetic group (143, 144). However, ADA recommends the use of Clopidogrel in very high-risk diabetic patients as an alternative therapy. The CURE study evaluated Clopdiogrel plus aspirin versus aspirin alone in the patients with unstable angina in more than 12,000 subjects (145). Dual anti-platelet therapy showed beneficial effects in the diabetic subgroup but the results were found to be borderline significant. However one should note that acute event rates are usually much higher in the diabetic subjects compared to non-diabetic subjects. Both American College of Cardiology as well as American Heart Association recommend the addition of Clopidogrel to the use of aspirin for management of unstable angina. There is great need to understand the clinical complications associated with hyperactive platelets. This is especially true in diabetic patients and this phenomenon may be responsible for the rebound occurrence of acute vascular events following withdrawal of anti-platelet therapy. In several countries, combinations of aspirin and Clopidogrel are available. Data from well-conducted clinical studies using these combination drugs especially in diabetic subjects are not available.

There are several ADP antagonists such as Prasugrel, Ticagrelor, Cangrelor and Elinogrel under clinical evaluations. Prasugrel (Efient) is a selective and irreversible inhibitor of ADP-induced platelet aggregation and is indicated for the secondary prevention of acute thrombotic events (148). In India several cardiologists recommend daily use of Clopidogrel or Aspirin or a combination of these drugs, for secondary prevention of acute vascular events in patients who have undergone bypass surgery or PCI. One has to be careful before recommending intensive anti-platelet therapy as it may lead to excessive bleeding. The risk-benefit ratios should be thoroughly assessed before recommending aggressive anti-platelet therapy (Personal opinion).

In a study done in Korea by researchers, triple (Cilostazol 100mg twice a day, Clopidogrel, 75 mg/day with aspirin), versus dual anti-platelet therapy (Clopidogrel

/aspirin) was tested in patients with acute myocardial infarction undergoing percutaneous coronary intervention (PCI). In this study with 2074 diabetic patients undergoing PCI with drug eluting stents, the group receiving triple drug therapy had significantly lower incidence of death and major adverse cardiac events (146). Cilostazol is currently recommended for use in patients with severe and disabling symptoms and there is insufficient evidence to recommend routine use of new agents such as Picotamide in patients with vascular disease (147). There is a great need for clinical evaluation, research and development of newer drugs to address the needs of patients with peripheral vascular disease. Review of management of diabetes related complications in this group of patients are beyond the scope of this article.

Several studies have evaluated the effectiveness of various GP11b/111a antagonists (134). At present there are three drugs in this category that are approved for clinical use; Abciximab, Eptifibatide, and Tirofiban. A meta analysis of 6 clinical trials shows that this class of drugs significantly reduce mortality at 30 days in diabetic patients. Current guidelines support the use of these drugs in patients with acute coronary syndromes, especially those with diabetes. Other thromboxane antagonists such as Ramatroban, Ridogrel and S18886 are under clinical trials. Development of newer drugs for anti-platelet and antithrombotic therapies is a complex process. Platelets have multiple mechanisms for activation. Similarly several proteins play a critical role in the activation of coagulation cascade. There is no drug that prevents all the activation mechanisms of this system; Moreover risk outweighs the benefit if we develop a drug that inhibits all the activation mechanisms. Therefore, there is a great need to develop newer safer drugs and their combinations, thorough evaluation and to have access to Point-of-Care monitoring capabilities for such customized therapy.

12. CONCLUSIONS

Type-2 diabetes is reaching epidemic proportions worldwide. According to WHO estimates, it will double in the most of the countries in next decade. Individuals with diabetes have a 2-4 fold higher risk of developing acute cardiovascular and cerbrovascular events. Patients with diabetes seem to have hyperactive thrombotic state. There is ample experimental evidence to suggest that they have circulating platelets with increased sensitivity to activating agents. Effective management of platelet function could reduce the acute vascular events significantly. Aspirin and Clopidogrel are the choice of drugs available for secondary and primary prevention of acute vascular events. In recent years there is considerable concern about the aspirin and Clopidogrel resistance. Aspirin resistance is a rare phenomenon in healthy individuals. Observed aspirin resistance in clinical situations may be the result of altered sensitivity of platelets to the action of other stimulants. Furthermore, as demonstrated by Rao *et al* in their studies, endogenous agonists such as epinephrine, and ADP potentiate the action of other platelet stimulants (94-101). There is a great need for the development of Point-of-Care devices for monitoring anti-platelet therapy. Anti-platelet and antithrombotic therapy should be monitored in clinical situations and appropriate customized treatment plans should be developed to suit the needs of individual patients. The development of newer anti-platelet agents and antithrombotic agents should be prioritized. This strategy will provide safer and effective drugs and will be the choice of the future anti-platelet regimens, for the better management of individuals with thrombotic tendency

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