Platelet hyperactivity in type 2 diabetes: role of antiplatelet agents

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Abstract

Type 2 diabetes mellitus increases atherothrombotic risk. Platelets in individuals with diabetes show increased activity at baseline and in response to agonists, ultimately leading to increased aggregation. Increased expression of platelet surface adhesion molecules and receptors, enhanced production of thromboxane and thrombin and disturbances in platelet calcium homeostasis are well documented. As intra-arterial thrombi are initiated by platelets, strategies to limit acute thrombotic events have largely focused on antiplatelet agents. Aspirin remains the cornerstone of antiplatelet therapy but appears to have limited benefit in diabetes. Use of thienopyridines and platelet glycoprotein IIb/IIIa receptor inhibitors has been shown to benefit high-risk patient populations. This review summarises the different platelet abnormalities characterised in diabetes and the role of currently used antiplatelet agents.

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Key words: antiplatelet agents, haemostasis, platelets, platelet hyperactivity, type 2 diabetes.

Introduction

A search of PubMed (Medline) was performed starting from the year 1975 until the present, using the following terms either singly or in combination: type 2 diabetes mellitus, diabetes, cardiovascular risk, hypercoagulability, prothrombotic, haemostasis, haemodynamic derangements, procoagulant, platelet abnormalities, platelet activation, platelet dysfunction, platelet hyperactivity, antiplatelet agents, aspirin, clopidogrel, thienopyridine and glycoprotein IIb/IIIa inhibitors. Papers were considered on the basis of relevance to platelet abnormalities in type 2 diabetes and currently used antiplatelet agents.

The current global diabetes “pandemic” relates mainly to type 2 diabetes mellitus; its prevalence, which was estimated to be 150 million people in 2000, is set to rise to 220 million in 2010, and 300 million in 2025. Individuals with type 2 diabetes have a 2- to 4-fold increased risk of developing atherosclerotic cardiovascular disease. Studies have demonstrated worse outcomes among patients with diabetes following acute coronary syndromes and coronary revascularisation procedures. Indeed, cardiovascular mortality in people with diabetes without a history of myocardial infarction is comparable to mortality in non-diabetic subjects with previous myocardial infarction. Accordingly, diabetes has been classified as a coronary “risk equivalent”.

Atherothrombosis secondary to endothelial dysfunction, inflammation, thrombosis, oxidative stress, dyslipidaemia and haemodynamic shear stress plays an important facilitative role in the long-term development of vascular disease in patients with type 2 diabetes. The primary cause of mortality in the majority of patients with diabetes is, however, atherothrombosis, often presenting as acute coronary syndromes. Acute coronary syndromes are precipitated by the ischaemic effect of an occlusive intracoronary thrombus which develops over a ruptured atheromatous plaque. Platelet hyperactivity (in combination with abnormalities in coagulation and fibrinolysis), which is characteristic in diabetes, undoubtedly contributes to this as platelets play a pivotal role in initiating and sustaining thrombi within vessels. Of concern is the observation that excess cardiovascular risk in patients with diabetes persists despite controlling for traditional factors, including hypertension, smoking, hypercholesterolemia and physical inactivity. This suggests that other factors, such as a prothrombotic state, may be contributory. After acute coronary syndromes, patients are treated with the same antiplatelet drug regimen at the same doses, irrespective of the presence of diabetes. Data show that outcomes in diabetic patients on standard antiplatelet therapy for secondary prevention are significantly worse, compared to their non-diabetic counterparts. This review will examine the mechanisms of platelet hyperactivity associated with diabetes and the commonly used classes of antiplatelet agents.

Platelet hyperactivity in type 2 diabetes

The physiological mechanism of platelet-dependent thrombus formation is summarised in figure 1. Platelet activation is
Table 1. Summary of platelet abnormalities in diabetes

| Increased production of thromboxane A2 from arachidonic acid |
| Increased expression of platelet surface adhesion molecules such as CD31, CD49b, CD62P and CD63, leading to increased platelet activation |
| Increase in platelet-dependent thrombin generation |
| Increased surface expression of platelet surface receptors such as P-selectin, GP Ib and GP IIb/IIIa, possibly due to non-enzymatic glycation of the receptor proteins. GP Ib mediates binding to vWF and GP IIb/IIIa binds fibrinogen – important steps in thrombogenesis |
| Reduced vascular synthesis of the anti-aggregants PGI2 and NO, shifting balance towards aggregation and vasoconstriction. In addition, platelets appear to be less sensitive to the effects of these agents |
| Disordered calcium homeostasis occurs in platelets, which can affect platelet shape change, secretion, aggregation and thromboxane formation |
| Decreased platelet insulin receptor number and affinity is observed. Insulin is thought to reduce platelet responses to the agonists ADP, collagen, thrombin, arachidonic and PAF |
| Glycation of circulating LDL can occur, rendering platelets hypersensitive. Glycated LDL causes an increase in intracellular calcium concentration and platelet NO production, as well as inhibition of the platelet membrane Na+/K+-ATPase activity |

**Key:** GP = glycoprotein; vWF = von Willebrand factor; ADP = adenosine diphosphate; TxA2 = thromboxane A2; PAF = platelet activating factor; PF4 = platelet factor 4

**Note:** Thrombin is also generated during physiological blood coagulation via interaction of tissue factor and factor VIII

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**Increased thromboxane production**

Thromboxane A2 (TXA2) is a potent platelet activator whose synthesis is suppressed by aspirin. Abnormalities in TXA2 production were among the earliest characterised abnormalities in platelets of diabetic subjects. The production of TXA2 in patients with preserved renal function can be measured by the urinary excretion of 11-dehydro-TXB2, its major enzymatic metabolite. Excretion of this metabolite was found to be significantly raised in patients with type 2 diabetes.20-24 Tight metabolic control with insulin therapy resulted in a 50% reduction in 11-dehydro-TXB2 excretion. Aspirin reduced the excretion of this metabolite and discontinuing aspirin reversed this effect, thus confirming its platelet origin.26

**Increased expression of adhesion molecules and receptors**

A variety of adhesion molecules are pre-stored in intraplatelet granules and expressed on the platelet surface on activation. Platelets from patients with type 2 diabetes, compared to controls, were found to have increased expression of adhesion molecules CD31, CD36, CD49b, CD62P and CD63 when assessed by flow cytometry.27 Improved glycaemic control over a three-month period led to a significant decline in their expression.27 Platelets in diabetic patients have increased surface expression of GP Ib and GP IIb/IIIa.28

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**Figure 1. Flow chart of physiological platelet-dependent thrombogenesis.** Arterial wall damage triggers a cascade of events starting with platelet adhesion to the damaged vessel area, which then leads to platelet activation, aggregation and finally thrombus formation.
GP Ib mediates binding to von Willebrand factor (vWF) – an important step in platelet-dependent thrombogenesis (see figure 1). Increased expression of GP Ib/IIa on platelet surfaces leads to enhanced fibrinogen binding and subsequently platelet cross-linking and thrombogenesis. It must be noted that fibrinogen levels per se can be raised in association with type 2 diabetes.

Other effects of hyperglycaemia
Acute in vivo hyperglycaemia results in platelet activation in type 2 diabetes. Furthermore, a link between hyperglycaemic spikes and incidence of ischaemic events has been demonstrated. In a study of patients with type 2 diabetes, shear stress-induced platelet activation, P-selectin expression on platelet surface and 11-dehydro-TXB2 excretion were increased after hyperglycaemic clamping, whereas no changes were observed after euglycaemic clamping. Hyperglycaemia also causes non-enzymatic glycation of platelet membrane proteins resulting in changes in protein structure and conformation, as well as alterations of membrane lipid dynamics. This in turn can lead to enhanced expression of certain crucial platelet receptors, for instance, P-selectin and GP Ib/IIa, thus altering platelet activity. Glycation of circulating low-density lipoproteins (LDL) can render platelets hypersensitive. Glycated LDL causes an increase in intracellular calcium concentration and platelet nitric oxide (NO) production, as well as inhibition of the platelet membrane Na+/K+-adenosine triphosphatase (Na+/K+-ATPase) activity. In parallel, other lipid abnormalities characteristic of type 2 diabetes such as elevated levels of triglyceride, high-density lipoprotein (HDL) and small, dense LDL can also affect platelet function by interfering with membrane fluidity and intracellular systems.

Platelet-endothelial cell interactions
In healthy vessels, prostacyclin (PGI2) and NO combine to prevent platelet adherence to endothelium and platelet aggregation. These anti-aggregants are released continually by healthy endothelium, but their synthesis is increased in the vicinity of aggregating platelets in response to agents such as thrombin and bradykinin. This is thought to limit the growth of the platelet plug to the area of denuded vessel endothelium. Reduced vascular synthesis of the anti-aggregants PGI2 and NO by the endothelium occurs in diabetic subjects, shifting the balance towards aggregation and vasoconstriction. Additionally, platelets from diabetic individuals are less sensitive to the effects of PGI2 and NO.

Role of calcium and magnesium
Platelets in individuals with type 2 diabetes show disordered calcium homeostasis. This may contribute significantly to hyperactivity, since intraplatelet calcium regulates a variety of activities, including platelet shape change, secretion, aggregation and thromboxane formation. Magnesium deficiency, which can occur in diabetes, has been linked with platelet hyperaggregability and adhesiveness. Intravenous magnesium supplementation was found to reverse these changes in one study.

Role of insulin
Normal platelets have been shown to be targets of insulin action as they retain a functional insulin receptor capable of insulin binding and autophosphorylation. Insulin is generally thought to reduce platelet responses to the agonists adenosine diphosphate (ADP), collagen, thrombin, arachidonate and platelet-activating factor. Decreased platelet insulin receptor number and affinity occur in subjects with type 2 diabetes, which suggests that reduced insulin sensitivity may account for platelet hyperactivity in this condition.

Platelet-dependent thrombus formation
Arterial injury models which simulate in vivo coronary artery rheology have been used to measure platelet-dependent thrombus formation. These models provide a measure of actual thrombus which is the end point of haemostatic functions, including platelet activity. A study by the authors of this review demonstrated increased thrombogenicity in patients with type 2 diabetes compared to non-diabetic controls despite treatment with aspirin and other risk factor therapies. Another study by the same group demonstrated an improvement in thrombogenic potential in diabetic subjects with glycaemic control.

Role of antiplatelet agents
A variety of antiplatelet agents are in use which act via different platelet receptors and pathways. There is a clear benefit of antiplatelet agents in the prevention of occlusive vascular events in those at risk. However, there is considerable variability in individuals' responsiveness to antiplatelet agents. Multiple genetic, iatrogenic and environmental factors influence platelet responsiveness. The four most commonly used antiplatelet agent groups are discussed.

Aspirin
Aspirin selectively acetylates the cyclo-oxygenase (COX) enzyme in platelets, inactivating its COX-1 isoform, which results in suppression of TXA2 production and thus inhibition of platelet aggregation. This action is irreversible since new platelets are continuously being produced, a regular regimen of aspirin is essential. Low-dose aspirin (75–150 mg daily) is both efficacious and cost-effective and is therefore the bedrock of secondary prevention of cardiovascular disease. It confers a 19% relative risk reduction for arterial disease as a whole. Aspirin withdrawal can lead to recurrence of acute coronary syndromes.

A meta-analysis by the Antiplatelet Trialists' Collaboration found doses of 75 to 150 mg to be of optimal efficacy, with the smallest incidence of bleeding. Higher doses (500 to 1,500 mg daily) caused a higher incidence of side effects with no added benefit. The above analysis found that the benefit of aspirin may be limited in patients with diabetes mellitus. Among 4,961 patients with diabetes in nine trials, antiplatelet therapy was associated with a non-significant 7% proportional reduction in vascular events, which was only about one quarter of the observed benefit overall. In the population as a whole, there was a 26% reduction in
non-fatal myocardial infarction or death from coronary heart disease (p<0.0001) and a 30% proportional decrease in fatal or non-fatal ischaemic stroke (p<0.0001) with antiplatelet therapy. It must be noted that this meta-analysis included trials evaluating different antiplatelet agents, but aspirin was the most widely studied. Furthermore, a recent study involving a cohort of nearly 2,500 patients with acute coronary syndromes found through multivariable analyses that aspirin was not associated with significant mortality benefit in patients with diabetes.

The role of aspirin in primary prevention is still contentious. There is evidence that aspirin in individuals without known coronary disease, but with risk factors such as diabetes, reduces the risk of adverse events by up to 28%. Accordingly, the American Diabetes Association and the American Heart Association recommended primary prevention with low-dose aspirin in patients with diabetes who are classified as being at high risk of cardiovascular events (the ten-year risk of coronary heart disease in such patients is estimated to be greater than 10%). However, the Primary Prevention Project trial found no benefit of aspirin in primary prevention in patients with diabetes, but demonstrated consistent benefit of aspirin in patients without diabetes but with other cardiovascular risk factors.

"Aspirin resistance"
The topic of aspirin resistance has been reviewed extensively. Biochemically, this refers to the inability of aspirin to inhibit platelet production of TXA2 and therefore platelet aggregation. Clinically, it is defined as the recurrence of thromboembolic events in patients prescribed therapeutic doses of aspirin.

Several possible causes for "aspirin resistance" have been proposed: reduced bioavailability of aspirin, alternative sources of TXA2 production, altered binding of aspirin to COX-1, alternative pathways of platelet activation, increased turnover of platelets, genetic polymorphisms of enzymes and receptors involved in the platelet activation pathway, drug interactions and tachyphylaxis. Although studies have reported the prevalence of "biochemical aspirin resistance", they have limitations: sample sizes were often small, poor adjustment was made for confounding factors, varying protocols and aspirin doses were employed and information on the relationship of "aspirin resistance" to clinical events was scant. Numerous other studies have investigated the relation of "aspirin resistance" to clinical vascular events. These reported a significant increase in vascular events in individuals demonstrating "aspirin resistance" in the laboratory. These studies too had several drawbacks: wide variations in testing and interpretation of "aspirin resistance", suboptimal assessment of compliance, performing a single assessment of platelet function thus making it difficult to assess stability over time, lack of agreement between different platelet function tests, variation in dose regimens, lack of statistical power and small number of incident clinical events.

Overall, the term aspirin resistance does not provide much information on the mechanistic pathways responsible for aspirin failure and therefore is of little use to clinicians. There is no consistent definition of the state and there are no standardised testing methods. It must be emphasised that there are several critical factors which influence clinical outcomes in individuals with diabetes and cardiovascular disease. Risk profiles of individual patients vary considerably and the pathophysiological mechanisms underlying each cardiovascular event may be different. It is possible in theory though, that aspirin may have little or no effect on many of the platelet and coagulation abnormalities associated with type 2 diabetes. Thus, the questions remain -- is aspirin resistance real? If so, is it more prevalent in patients with diabetes? Can it be diagnosed reliably by routine laboratory measures? And finally, can "aspirin resistance" consistently predict risk of clinical thrombotic events?

Thienopyridines
Clopidogrel is the most widely used thienopyridine derivative now that ticlopidine has been sidelined because of side effects. Clopidogrel induces irreversible alterations of the platelet receptor P2Y12 mediating inhibition of stimulated adenyly cyclase activity by ADP. It inhibits platelet aggregation by a mechanism different to that of aspirin and can thus, in theory, be expected to add to its effects. In fact, a recent study demonstrated that treatment with clopidogrel plus aspirin compared with aspirin alone for one month, provided significantly greater inhibition of platelet activity in diabetic patients. Multiple tests of platelet function were used in this study, including agonist-induced aggregometry, PFA-100, RPFA and flow cytometry. The efficacy of clopidogrel was found to be superior to that of aspirin, with a comparable safety profile, in a trial of stable patients with arterial disease. A subsequent subgroup analysis of 3,866 patients with diabetes from the same study demonstrated a much greater beneficial effect with clopidogrel in this group. The event rate per year was 15.6% in the 1,914 individuals randomised to clopidogrel and 17.7% in the 1,952 patients who received aspirin, with an absolute risk reduction of 2.1% (p=0.042). This study, however, was not designed to assess the effects of combined clopidogrel and aspirin therapy.

Another large study in patients with acute non-ST-segment elevation myocardial infarction found that combination therapy with aspirin and clopidogrel proved more beneficial than treatment with aspirin alone. Ischaemic cardiovascular events or cardiovascular death occurred in 16.5% of the patients treated with combination therapy compared to 18.8% of the patients treated with aspirin alone over a
Another American study have demonstrated clear benefit and in the 5,072 non-diabetic patients, from 2.6% to 1.9% with a reduction in mortality from 4.5% to 2.5% (p=0.031). A combined analysis of three trials showed that among the 1,262 diabetic patients, use of these agents was associated with significantly reduced rates of ischaemic events, including stent thrombosis, but with an increased risk of major bleeding. Of note, a particular benefit in event reduction was noticed in the subgroup of patients with diabetes.

Platelet glycoprotein IIb/IIIa receptor inhibitors

Platelet glycoprotein (GP) IIb/IIIa inhibitors, which are administered intravenously, have a rapid onset of action and short half-life. They are used primarily as adjunctive agents in patients with acute coronary syndromes and in those undergoing percutaneous coronary intervention. Different agents with varying molecular structures and pharmacological features are in use. The GP IIb/IIIa receptor binds fibrinogen, and as this is the principal mechanism behind platelet aggregation (see figure 1), these agents can impair platelet-dependent thrombus formation irrespective of the metabolic pathway responsible for initiating it. The benefits of GP IIb/IIIa inhibitors during percutaneous coronary intervention have been established. Patients with diabetes seem to accrue greater benefit with the use of these agents. A combined analysis of three trials showed that among the 1,262 diabetic patients, use of these agents was associated with a reduction in mortality from 4.5% to 2.5% (p=0.031) and in the 5,072 non-diabetic patients, from 2.6% to 1.9% (p=0.099). In a later meta-analysis of six large-scale trials, which included a total of 6,458 patients with diabetes and acute coronary syndromes, GP IIb/IIIa inhibitor therapy was associated with a significant mortality reduction at 30 days, from 6.2% to 4.6% (OR 0.74; 95% CI 0.59 to 0.92; p=0.007). Conversely, in the remaining 23,072 non-diabetic patients no survival benefit was found (3.0% versus 3.0%). Furthermore, the interaction between GP IIb/IIIa inhibitor use and diabetic status was found to be statistically significant (p=0.036). Bleeding risk is, however, increased with their use.

Dipyridamole

This is thought to work in part by inhibiting platelet cyclic-3',5'-adenosine monophosphate and cyclic-3',5'-guanosine monophosphate phosphodiesterase. A meta-analysis showed that dipyridamole, given alone or with aspirin, reduced stroke recurrence. Its role in prevention of acute cardiac events remains unproven. No special benefits have been reported in patients with diabetes.

Conclusion

A prothrombotic milieu, involving platelet hyperactivity, is present in individuals with type 2 diabetes, which may contribute to their increased risk of atherothrombotic events. Platelet adhesion, activation and aggregation are abnormal in individuals with diabetes and hyperglycaemia. An array of antiplatelet agents acting on various target points in platelets have been developed, but they do not appear to reduce completely the heightened thrombotic potential, even in combination regimes. Furthermore, there remains the uphill task of balancing clinical efficacy against bleeding risk. Large randomised trials specifically designed to study these aspects in the population with diabetes are lacking. Despite better understanding of the pathophysiological processes underlying platelet activation and therapeutic advancements, the morbidity and mortality from atherothrombosis in type 2 diabetes remain unacceptably high. This may become even worse with the projected global upsurge in obesity and type 2 diabetes. This dictates that we scrutinise platelets more closely and accelerate the search for more potent antiplatelet agents. It is perhaps time to turn up the volume.

Conflicts of interest statement

None declared.

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