

Antiplatelet Agents: All for One, One for All... Is it Still True?

Introduction

Platelets, small elements that circulate normally in the bloodstream, help the body to defend itself against bleeding and blood loss. They do this by sticking together and helping in the formation of a clot at the site of bleeding. Although this process of creating a blood clot, which is called thrombosis, is beneficial in a person with severe bleeding, it can also cause problems, particularly when a blood clot forms in the circulation of the heart or brain. A heart attack results when a blood clot interrupts or blocks blood flow to the heart, which starves the heart muscle of oxygen and causes death of heart muscle cells; the same process in the brain causes a stroke [1].

Under normal healthy conditions, the different parts of flowing blood (such as red and white blood cells and platelets) are unable to stick to the inner lining of blood vessels and cause a blockage that disrupts blood flow. However, when there is a buildup of fatty deposits (atherosclerosis) within blood vessels, the lining of the vessels (the endothelium) is less resistant to blood clotting [2]. A variety of stimuli, including high blood pressure, high blood sugar, and poisonous chemicals, like tobacco components may make atherosclerotic fat deposits (plaques) unstable. This is particularly true when plaques are rich in fat (cholesterol) and white blood cells (inflammatory cells). An unstable plaque may crack or rupture and expose its contents to flowing blood. In its own defense, the body attempts to heal this injury by forming a blood clot over the damaged area [3].

The formation of a blood clot occurs in several steps. First, platelets adhere to the plaque surface, in a process known as adhesion. They, then begin to stick together in a process known as aggregation. The growing platelet aggregate forms a surface on which the process of coagulation can occur: through a series of enzymatic reactions, red strands that mesh together to become a net-like substance (fibrin) form, linking platelets together, red blood cells are trapped within the fibrin meshwork, and a blood clot results [4]. It's clear that platelets play a key role in haemostasis and thrombosis. Multiple factors such as genetic polymorphisms, pathologic state and lifestyle are considered to be associated with platelet hyperactivity. Platelets are activated by a number of agonists and blood flow resulting in the expression and/or activation of surface receptors, secretion of vasoactive substances, adhesion, aggregation, and finally thrombus formation. These events are propagated by a process known as transmembrane signaling, which then serves to activate the platelet via a cascade of biochemical interactions [5].

It involves in multiple signaling molecules, each with various enzymatic activities and/or functions. Based on the signaling pathway of platelet activation, there are 4 considerable strategies for platelet function inhibition and anti-platelet targets:

- a. Inhibition of agonist generation;
- b. Receptors inhibition;
- c. G-protein inhibition; and
- d. Inhibition of enzymatic cascades.

Due to the problems existing in current anti-platelet agents, future new strategies for anti-platelet targets, agent-developing and treatment might probably include three aspects: from mono-target to multi-target, from mono-agent therapy to combination therapy and from clinically approved "non-antithrombotic" agents to discovery anti-platelet effect [6]. The major physiological function of platelets is in haemostasis, which is the maintenance of vessel integrity and cessation of bleeding upon injury. However, pathophysiologic conditions, platelet activation leads to a range of responses that play a critical role in arterial thrombosis and the inflammatory responses associated with this, including platelet aggregation, dense and a granule secretion and procoagulant activity [7]. Over the last decade, it has become clear that platelet activation represents a multistep process, involving distinct receptors and ligands, with the contribution of individual receptor-ligand interactions to the activation process dependent on the prevailing blood flow conditions [8]. With the development of extensive gene knockout studies and the availability of multiple receptors antagonists, the details of process of platelet activation have now become more and more clear. And many reviews have summarized this complex process from various aspects: platelet physiology, signal transduction, G protein and so on [9-11].

Factors associated with enhanced platelet activation

Epidemiological studies have linked platelet hyperactivity with an increased risk of vascular events. Even more convincing is the evidence from appropriately designed clinical trials, showing that antiplatelet agents decrease the risk of vascular events (myocardial infarction and stroke). These findings are compatible

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G Di Micco**Department of Internal Medicine, Italy*

***Corresponding author:** Gianluca Di Micco, Via San Domenico, "Buon Consiglio" Fatebenefratelli Hospital - Naples, Italy, Email: gdimicco@inwind.it

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with the known thrombotic action of platelets. However, it is difficult to reliably identify the 'high risk patient' as the absence of a reliable universally accepted marker of platelet activity.

Genetic polymorphisms

There is marked interindividual variation in platelet responsiveness, with some subjects displaying platelet hyperreactivity. This variation is for a large extent genetically controlled [12]. The polymorphisms of several platelet receptors have been considered to be associated with increased platelet aggregation and the variability in responses to anti-platelet agents which is multi-factorial and is not caused only by single gene mutations [13]. It is known that the platelet P2Y₁₂ receptor plays a key role in platelet activation. Recently, the H2 haplotype of the P2Y₁₂ receptor gene (P2RY12) has been found to be associated with maximal aggregation response to ADP and with increased risk for peripheral arterial disease. Moreover, carriers of the H2 haplotype may have an increased risk of atherothrombosis and/or a lesser clinical response to drugs inhibiting platelet function. In addition, at least three different isoforms termed PDE2, PDE3 and PDE5 isoenzymes have been identified in platelets, which means, there is individual variation in responses to such anti-platelet agents as dipyridamole and cilostazol acting by inhibition of PDE [14,15].

Pathologic state

Atherosclerosis and vascular events: The normal response of the platelet can be altered, either by increased pro-aggregatory stimuli or by diminished anti-aggregatory substances to produce conditions of increased platelet activation/aggregation and occur in active cardiovascular disease states both on a chronic (stable angina pectoris) and acute basis (acute myocardial infarction).

Hyperlipidemia: Platelet hyperreactivity can be assessed with multiple stimuli in multiple assays and is more likely to be present in subjects who have elevated fibrinogen, triglycerides, and low density lipoprotein (LDL) levels. The interaction of platelets with lipoproteins has been under intense investigation. Recently, observations suggest that LDL enhances platelet responsiveness. Several signaling pathways induced by LDL have been revealed in vitro, such as signaling via p38 mitogen-activated protein kinase and p125 focal adhesion kinase. In contrast to LDL, High density lipoprotein (HDL), consisting of two subtypes, HDL [2] and HDL [3], has opposing effects on platelet activation [16].

Diabetes: Diabetes is a well-recognized risk factor for atherosclerotic cardiovascular disease and in fact diabetes is associated with enhanced platelet activation. In particular, an altered platelet metabolism and changes in intraplatelet signaling pathways may contribute to the pathogenesis of atherothrombotic complications of diabetes. Diabetic patients have hyperreactive platelets with exaggerated adhesion, aggregation and thrombin generation. In summary, the entire coagulation cascade is dysfunctional in diabetes [17]. A variety of mechanisms may be responsible for enhanced platelet aggregation. First, hyperglycemia may be responsible for nonenzymatic glycation of platelet glycoproteins, causing changes in their structure and conformation, as well as alterations of membrane lipid dynamics. Secondly, hyperglycemia-induced oxidative stress is responsible for enhanced peroxidation of arachidonic acid to form biologically

active isoprostanes, which represents an important biochemical link between impaired glycemic control and persistent platelet activation. Moreover, increased oxidative stress is responsible for activation of transcription factors and expression of redox-sensitive genes leading to a phenotypic switch of endothelium toward an adhesive, pro-thrombotic condition, initial platelet activation, adhesion and subsequent platelet aggregate formation. In addition, greater reduction in platelet activity observed with osiglitazone may be just related to reduced oxidative stress and a possible direct PPAR γ mediated effect on platelet function. Third, high glucose levels enhanced platelet reactivity to agonist stimulation, through elevated osmolality. This occurred via superoxide anion production, which enhanced platelet P-selectin expression (secretion), and PKC signalling, which enhanced TRAP-induced fibrinogen binding (aggregability). Finally, studies have also showed that food intake enhances thromboxane receptor-mediated platelet activation in type 2 diabetic patients but not in healthy subjects [18-21].

Hypertension: Platelet activation is involved in the pathogenesis of the thrombotic complications of hypertension. There is a stepwise increase in platelet activation indices, despite similar platelet counts, with increasing severity of hypertensive disease. This may contribute to the pathogenesis of thrombosis-related complications in hypertension. Recent studies identified the association of extreme blood pressure elevation with platelet P-selectin and fibrinolytic markers in high risk patients with severe hypertension [22].

Other diseases: Epidemiological evidence suggests that hyperhomocysteinemia may lead to increased platelet activation and is an independent risk factor for arterial thrombotic diseases such as acute myocardial infarction, stroke, peripheral ischemic occlusive disorders as well as venous thromboembolism. In addition, metabolic syndrome is also associated with enhanced platelet activation. In overweight and obese outpatients, metabolic syndrome risk factors parallel to some extent platelet responsiveness to leptin [23,24].

Lifestyle

No doubt, lifestyle habits such as exercise, smoking, diet, and alcohol consumption may have significant influence on cardiovascular disease and platelet function. Human lifestyle or physical activities have diverse effects on platelet reactivity. There have been abundant studies of the effects of exercise, weight loss, dietary lipids (especially n-3 PUFA), smoking, alcohol, and psychosocial stress on platelet activation. Available evidence from studies support lifestyles that adopt strategies to lose weight, stop cigarette smoking, engage in regular moderate exercise and relaxation, and regularly consume light-to-moderate alcohol and fatty fish should significantly reduce platelet reactivity. Both epidemiological studies and clinical trials indicate that the very long chain n-3 fatty acids lower thrombotic tendency and risk of heart disease. Other polyunsaturated fats and monounsaturated fat appear to have antithrombotic properties, but further studies are indicated. In a limited number of clinical and laboratory studies, vitamin E has been shown to decrease platelet aggregation. Flavonoids and isoflavones appear to inhibit platelet aggregation at pharmacologic concentrations only. Nutritional status frequently declines with aging and may exacerbate the

already increased risk for thrombosis [25].

Platelet activation needs the induction of agonists, and most agonists can act synergistically in platelet aggregation. Therefore, inhibition of agonist generation may be a good strategy for anti-platelet targets. However, most agonists act many other physiological function, not just activating platelet. Until now, only TXA₂ generation and thrombin inhibitors and have been applied in clinic, such as Aspirin and Argatroban; for other agonists such as ADP and collagen, they may not be proper as anti-platelet targets [26].

Receptors inhibitors

PAR1 and/or PAR4 antagonists: Thrombin activates human platelets through proteolytic activation of two protease-activated receptors (PARs), PAR1 and PAR4. RWJ-56110, a potent synthetic PAR1 antagonist, inhibited platelet aggregation caused by a low concentration of thrombin, but lost its effectiveness when higher concentrations of thrombin were used as stimulators. However, other studies showed that RWJ-58239, another PAR1 antagonist, hindered the thrombotic activity in nonhuman primates even in the presence of an active PAR4 receptor. This shows that PAR1 antagonists have therapeutic potential in the prevention of arterial thrombosis [27].

P2Y1 receptor antagonists: The P2Y1 receptor can be competitively and selectively antagonized by A3P5P, A3P5PS, and A2P5P compounds, and also by MRS2179 and MRS2279. As stated previously, P2Y1 deficient mice, as well as, wild type mice treated with P2Y1 receptor antagonists, have shown resistance to thromboembolism and significantly reduced arterial thrombus formation. Based on these findings we can conclude that P2Y1 receptor antagonists could serve as effective antithrombotic agents [28].

P2Y12 receptor antagonists: It has proved P2Y12 receptor plays a very vital role in the process of platelet activation and thrombus formation. While irreversible platelet inhibitors, such as Aspirin and Clopidogrel, have limited anti-thrombotic efficacy in the clinic, due to their bleeding risk, reversible antagonists of the P2Y12 receptor namely, AR-C66096, ARC67085, and AR-C69931MX become good anti-thrombotic agents, which have proven efficacy in vitro platelet functional assays and in animal models of thrombosis [29].

GPVI inhibitors: Thrombus formation after atherosclerotic plaque rupture critically involves the platelet collagen receptor glycoprotein (GP) VI. EXP3179, an active metabolite of the angiotensin II type 1 (AT1)-receptor antagonist Losartan, acts as a specific inhibitor of the platelet collagen receptor GPVI independent of AT1-receptor [30].

5-HT (2A) receptor antagonist: It is widely accepted that antiplatelet therapy is effective for secondary prevention of atherosclerotic vascular diseases. With double-blind, controlled clinical-pharmacological study to investigate the antiplatelet efficacy of sarogrelate, a selective 5-hydroxytryptamine (5-HT(2A)) receptor antagonist, in patients with ischemic stroke, results showed that sarogrelate treatment inhibited platelet aggregation dose-dependently in patients with ischemic stroke, as judged by a new assessment system employing combinations of 5-HT and epinephrine as agonists [31].

G-protein inhibitors

As Gq, G12/13, Gi and Gs integrate the effects of various platelet stimuli acting through their GPCRs, interference with signaling pathways downstream of GPCRs appears to be a promising strategy to develop antiplatelet agents with higher efficacy than those that block only individual platelet stimuli or their individual receptors. Because all 4 G protein-mediated signaling pathways show some level of redundancy, inhibition of 1 of the pathways should still be safer than blocking the common end point of platelet activation [32].

Enzyme inhibitors

TS inhibitors: Apart from COX inhibitors and TP receptor antagonists, TS inhibitors can also prevent the effects of TXA₂. Conversely, they result in the accumulation of prostaglandin H₂ (PGH₂), which by activating the TP receptor undermines their anti-platelet effects. Therefore, simultaneous antagonism of thromboxane receptor and inhibition of thromboxane synthase would be desirable as they would cause blockade of the effects of PGH₂ on TP receptor and interfere with TXA₂ generation. Studies have confirmed this hypothesis, by showing that drugs that can perform these functions lower the severity of myocardial ischemia [33].

PI3-Kinase inhibitors: The role of PI3-kinase in agonist-mediated GPIIb/IIIa activation and irreversible platelet aggregation has been well characterized. Studies performed with platelets obtained from PI3-kinase knockout mice demonstrated impaired and reversible platelet aggregation when stimulated with ADP and an added protection from ADP-induced thromboembolic events [34].

Other inhibitors: The increase in intracellular calcium levels is also essential for the activation of several enzymes like the classical isoforms of PKC, cytosolic phospholipase A₂ (cPLA₂), and proteases like calpain. Both calcium increases and PKC activation have been demonstrated to be essential for the induction of full platelet secretion as PKC inhibitors such as Ro 31-8220, or calcium chelators like BAPTA-AM block agonist-mediated granule secretion. In addition, pharmacological blockade of PLC activation, PKC activation, or intracellular calcium chelation inhibits agonist-mediated fibrinogen receptor activation and platelet aggregation [35].

Furthermore, since cyclooxygenase (COX) isozymes discovery, many papers and reviews have been published to describe the structural bases of COX inhibition, and to debate on the therapeutic and adverse effects of worldwide clinically used nonsteroidal anti-inflammatory drugs (NSAIDs), included COX-2 selective inhibitors (well known as Coxibs). COX-2 inhibition has been widely investigated, whereas the role of COX-1 in human pathophysiology is mostly not yet well ascertained. As time goes on, the cliché that the constitutively expressed isoform COX-1 is only involved in normal physiological functions, such as platelet aggregation, gastric mucosa protection and renal electrolyte homeostasis is going to be shattered. Low-dose aspirin, behaving as a preferential inhibitor of platelet COX-1, allowed to enlighten the role exerted by this isoenzyme in many mammalian cell types. Some studies would elucidate the most recent findings on selective COX-1 inhibition and their relevance to human pathology

such as cancer, neuro-inflammation, cardioprotection, fever and pain. It would also focus on the design and development of new highly selective COX-1 inhibitors, useful tools in pharmacological studies aimed at gaining a deeper insight of the role of COX-1 in human health and disease. Among the traditional NSAIDs, other than aspirin and indomethacin, only few examples of selective COX-1 inhibitors (SC-560, FR122047, mofezolac, P6 and TFAP) have been so far identified. The aim, is also to stimulate the development of novel drugs, which activity is COX-1 mediated.

Other meta-analysis have shown that selective COX 2 inhibitors are associated with a moderate increase in the risk of vascular events, as are high dose regimens of ibuprofen and diclofenac, but that high dose naproxen is not associated with such an excess. As differences between anti-inflammatory regimens are likely to be small, very large randomised trials will be needed if we are to identify which anti-inflammatory drug regimens minimise the overall burden of adverse gastrointestinal and cardiovascular outcomes.

Future direction of strategy for anti-platelet activation

Platelet activation is an integral component of the pathophysiology that leads to thrombotic and ischemic diseases such as myocardial infarction, cerebral stroke, and peripheral vascular disease. An ideal antiplatelet agent should specifically block thrombogenic platelet-dependent mechanisms in vascular diseases without interfering with normal platelet functions that are required in hemostasis and wound healing. Additionally, these agents should be free of any major adverse events. Although, several antiplatelet strategies have already been developed or are under preclinical or clinical investigation, none of the available antiplatelet drugs meet all of these criteria. The currently most widely used anti-platelet agents (such as aspirin, ADP receptor antagonists, and GPIIb/IIIa antagonists and phosphodiesterase inhibitors), are relatively well tolerated by patients towards treating ischaemic diseases. However, their limited efficacy in the setting of arterial thrombosis, unfavorable side effect, profile cost-to-benefit issues and "resistance" phenomenon substantiate the need for the development of newer and more efficacious anti-platelet and antithrombotic drugs [36]. Therefore, future new strategies for anti-platelet targets, agent-developments and treatment might probably include three aspects:

From mono-target to multi-target: For example, BM573, which blocks the TP receptor while inhibiting TS, inhibits AA induced platelet aggregation, prevents the formation of an occlusive thrombus, and reduces the occurrence of myocardial infarction (MI) without affecting heart rate or mean blood pressure. Not only because BM573 could prove to be useful in reducing platelet activation during acute coronary syndromes or coronary angioplasty [37]. In addition, developing some reversible agents like P2Y [12] receptor antagonist, BX 667, which has a wider therapeutic index than clopidogrel in experimental models of thrombosis [38].

From mono-agent therapy to combination therapy: Recent studies showed effects of triple antiplatelet therapy on platelet aggregation and P-selectin expression in patients undergoing coronary artery stent implantation. In conclusion, compared with dual antiplatelet therapy (aspirin plus clopidogrel), triple therapy (aspirin, clopidogrel, and cilostazol) after coronary stent placement

resulted in more potent inhibition of platelet aggregation induced by ADP and collagen. It suggests that triple therapy may be used clinically to prevent thrombotic complications after coronary stent placement [39]. Especially, for aspirin treatment failure (clinical aspirin resistance), consideration should be given to replacing aspirin with (or adding aspirin to) another anti-platelet agents or combination with two or more anti-platelet agents that inhibits other pathways of platelet agents or combination with two or more anti-platelet agents that inhibits other pathways of platelet activation and aggregation (ADP-receptor blocker such as clopidogrel or a phosphodiesterase inhibitor such as dipyridamole) [40].

From clinically approved "non-antithrombotic" agents to discovery anti-platelet effect: As platelet activation is induced and enhanced by many factors especially some pathologic states such as diabetes, hypertension and hyperlipidemia, more and more antihypertensive, anti-lipid and other categories agents have been found with direct or indirect antiplatelet effect, such as Nimodipine, Verapamil, ACE inhibitors, Statin and so on. However, some other agents might adversely interact and undermine the effectiveness of anti-platelet agents (ibuprofen and atorvastatin). Finally, considering the actual globalization, multiple chinese herbs have been reported with apparent inhibitory activity on human platelets. Actually, in oriental countries like China, such herbs have been widely used for several hundreds of years to treat thrombotic and ischaemic diseases. As herbs possess multiple constituents, their anti-platelet and antithrombotic mechanisms are quite complicated and still unclear [41,42].

Antiplatelet therapy is the cornerstone of the pharmacologic management of patients with vascular disease, including acute coronary syndrome, brain and peripheral vascular emergencies. Over the last years, several studies have evaluated old and new oral or intravenous antiplatelet agents in ACS patients. In particular, research was focused on assessing superiority of two novel platelet ADP P2Y12 receptor antagonists (prasugrel and ticagrelor); in fact prasugrel and ticagrelor have been shown to be adequate P2Y12 antiplatelet therapy alternatives to clopidogrel in the management of patients with ACS. While prasugrel and ticagrelor have both been shown to clinically improve platelet inhibition and significantly reduce the incidence of stent thrombosis, compared with clopidogrel therapy, both increase the risk of a significant bleeding incident. Both ticagrelor and prasugrel have been shown to be appropriate and effective treatment alternatives for ACS patients who display clopidogrel treatment resistance or failure. It is also true, that some studies clearly show the powerful dynamism of ticagrelor in a subset of patients, such as diabetics; in fact the higher potency and selectivity of ticagrelor are strongly underlined by difference of level of platelet reactivity inhibition achieved by prasugrel and ticagrelor loading dose in diabetic acute coronary syndrome patients undergoing PCI [43].

Conclusion

Treatment with aspirin and a P2Y12 inhibitors still remains the most common protocol used in patients with cardiovascular disorders. But the overall effect of such treatment on all-cause mortality is unknown. In the Dual Antiplatelet Therapy Study, continuation of dual antiplatelet therapy beyond 12 months after coronary stenting was associated with an unexpected increase in

non-cardiovascular death. In view of the potential public health importance of these findings, we aimed to assess the effect of extended duration dual antiplatelet therapy on mortality by doing a meta-analysis of all randomised, controlled trials of treatment duration in various cardiovascular disorders [44].

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