Print ISSN 2319-2003 | Online ISSN 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

doi: 10.5455/2319-2003.ijbcp20140402

Review Article

Antiplatelet therapy: present status and its future directions

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Received: 23 January 2014
Accepted: 02 February 2014

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ABSTRACT

Anti-platelets drugs play an important role in the prevention or treatment of cardiovascular diseases e.g. coronary artery disease, stroke, etc., which cause high mortality and morbidity in the present day world. These drugs either inhibit the platelet activation, aggregation or other signaling pathways, thereby inhibiting the clot formation. The anti-platelet drugs currently used are aspirin, ADP receptor inhibitors (ticlopidine and clopidogrel)and glycoprotein (GP)IIb/IIIa inhibitors (abciximab, tirofiban and eptifibatide). Aspirin was and still continues to be the main anti-platelet therapy. A combination regimen of aspirin and clopidogrel is commonly used for the prevention of platelet activation, thrombosis and stroke. However, many of the current anti-platelet drugs face limitations due to narrow therapeutic window and limited efficacy. The four possible targets for novel anti-platelet action are: Inhibition of agonist generation, receptor inhibition, G protein inhibition and inhibition of enzymatic cascades. Newer P2Y12 antagonists e.g. prasugrel, ticagrelor, cangrelor, etc., have better efficacy and low bleeding risk. The thrombin receptor (PAR1 and 4) inhibitors are said to decrease the hemorrhagic complications. Drugs which inhibit TXA2 Synthase or TXA2 receptor are also promising in their anti-platelet action. Another novel group is of collagen receptor antagonists such as GPVI antagonists, GPIb receptor antagonists, etc. The other targets being explored are von Willebrand Factor antagonists, platelet Gq antagonists, etc. However, there still lies a bundle of unresolved issues regarding the efficacy and safety, optimal dosage, administration requirements, combination therapy, clinical evaluation, cost-effectiveness, and the resistance phenomena of these drugs.

Keywords: Anti-platelets, Thrombin receptor antagonists, Collagen receptor antagonists, P2Y12 antagonists

INTRODUCTION

Anti-platelet drugs are the cornerstone in the treatment of cardiovascular diseases e.g., coronary artery disease, stroke, etc., which continue to be the most common cause of mortality and morbidity in the industrialized world.[1] Platelets play a key role in thrombosis and anti-platelet therapies may prevent as well as treat thrombotic diseases.[2] The major event leading to acute coronary syndrome (ACS) is thrombosis, which is caused by either the rupture or erosion of an atherosclerotic plaque. In this scenario, platelets and thrombin are the key players. Hence understanding the physiology of platelet activation, adhesion and aggregation is of paramount importance in the treatment of ACSs. Various studies have shown that anti-platelet therapy imparts the mortality benefit and also prevents the recurrent episodes of ischemia.[3]

Platelet activation and aggregations is a complex process comprising of multiple transmembrane signaling pathways and molecules with enzymatic activities and/or function after exposure to the platelet agonists.[3] The major chunk of anti-platelet drugs that are undergoing development target either the receptors present on the surface of platelets or enzymes in platelets. The first ever anti-platelet drug was aspirin, which inhibits the cyclo-oxygenase-1 enzyme. Though many new approaches to develop newer agents are being looked into, but still, aspirin is and will continue to enjoy the mainstay therapy status for the anti-platelet action, in the years to come. In spite of all the benefits which these anti-platelet drugs provide, they are laden with issues related to their use like narrow therapeutic window and limited efficacy, thereby limiting their general use. Currently, a regimen comprising of a combination of aspirin and clopidogrel is considered to be the standard treatment for preventing the platelet activation and aggregation and eventually, thrombosis and stroke. However, since the currently used anti-platelet drugs are fraught with problems such as resistance, optimal dosage, safety issues, etc., the future strategies for the development

of newer anti-platelet drugs and treatment regimens are being looked into.[1]

NORMAL PHYSIOLOGY OF PLATELET AGGREGATION

Platelets play a central role in maintaining hemostasis, but are also involved in athero-thrombotic diseases. Genetic polymorphisms result in large variability in platelet responsiveness to activation signals.[1,3] Platelets act as vascular sentries, monitoring the integrity of vascular endothelium. They have various receptors on their surface which participate in thrombotic events, once this integrity is compromised (Figure 1).[4] The sequences of events leading to the clot formation are described below (Figure 2). When the blood vessel wall is damaged, platelets are exposed to sub-endothelial structures e.g., collagen.[5] The interaction between collagen and platelets takes place through the two major collagen receptors namely integrin a2b1 and glycoprotein (GP) VI, to which the platelets bind. In high-shear conditions of arterioles, for effectively adhering, platelets require another receptor of collagen, i.e., GPIb which form a complex with factors V and IX along with its main ligand, von Willebrand Factor (vWF).[6] Binding of platelets through its direct collagen receptors triggers tyrosine kinase activity and induce the platelet activation cascade. This induces the conformational change in the platelets, causing the release of chemical mediators such as ADP, TXA2, 5HT, PAF, and thrombin. These mediators bind to their specific receptors present on the outer membrane of the resting platelets, which are circulating nearby the activated platelets. They as a result get activated and start to aggregate. The ultimate result of these signaling mechanisms is the increase in cytosolic calcium and decrease in cAMP in the platelet which causes the release of mediators such as ADP, 5HT & TXA2, activation of GPIIb/IIIa receptors that bind fibrinogen resulting in cross linking of platelets and their subsequent aggregation. Finally, the coagulation cascade is stimulated by the tissue factors released from the injured vessel wall, along with the mediators released from platelets and ultimately result in formation of thrombin. Thrombin, or activated factor II, being a serine protease, hydrolyses fibrinogen to fibrin & forms a plug.[4-6]

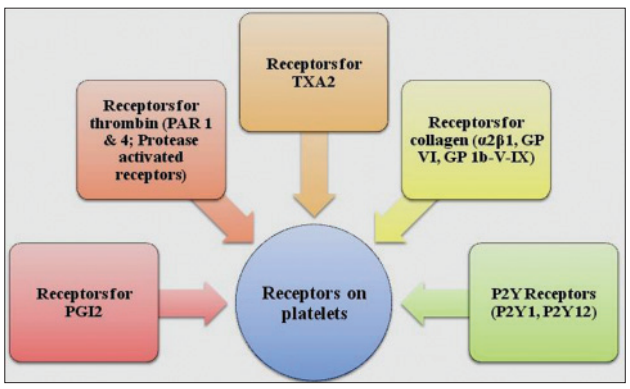

Figure 1: The receptors present on the surface of platelets.

CURRENTLY AVAILABLE ANTI-PLATELET DRUGS

If this process in excessively activated it will lead to thrombotic disorders and may even cause embolisms. In order to deal with such an abnormality, we need drugs that have an anticoagulant effect. The platelets provide the initial hemostatic plug at the sites of vascular injury, & anti-platelet drugs inhibit them in one way or other, thereby not allowing them to aggregate and hence the clot formation cannot take place.[2]

The anti-platelet drugs that are in use are: Aspirin, ADP receptor inhibitors (ticlopidine, clopidogrel) and GPIIb/IIIa inhibitors (abciximab, tirofiban & eptifibatide).[7]

Aspirin

It inhibits the enzyme cyclo-oxygenase-1, thereby blocking the thromboxane-mediated pathway. The effect of aspirin on platelets is permanent; lasting for the life of the platelet, i.e., 7-10 days. Thereby, there will be a cumulative inhibitory effect on platelets when aspirin is given on a daily basis. The anti-platelet effect of aspirin can be seen at low doses as much as 160 mg of aspirin daily, as it can effectively and completely inhibit COX-1 enzyme of platelets, even at such low doses. Higher doses are less efficacious and more toxic, with an increased risk of bleeding. It reduces the risk of death from cardiovascular causes, new myocardial infarction (MI) and recurrent ischemia by approximately 40%, as shown in several trials.[7] Efficacy has been demonstrated within a wide range of doses although 75-350 mg is the most used.[8-10]

ADP Receptor inhibitors: P2Y12 inhibitors

These drugs inhibit ADP induced aggregation of platelets. Ticlopidine and clopidogrel, both, are irreversible

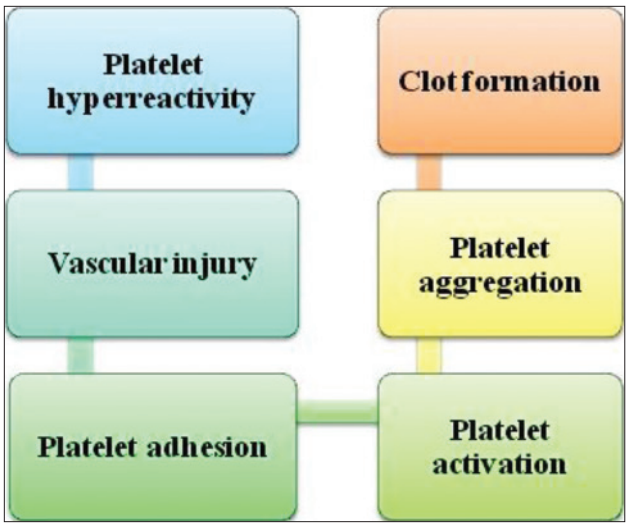

Figure 2: The sequence of events leading to the clot formation.

thienopyridine P2Y12 receptor antagonists currently available and have proven efficacy in reducing the risk of arterial thrombotic events.[7] Ticlopidine is a prodrug that needs to be first converted to its active metabolite by the action of cytochrome P450 enzyme. It permanently inhibits the P2Y12 receptor, forming a disulfide bond with the receptor. Maximal anti-platelet effect is seen in 8-11 days after starting the therapy. The most serious adverse effect is neutropenia, which occurred in 2.4% of recipients in premarketing trials. Hence frequent blood counts must be obtained during the first few months of therapy. Because of these life-threatening blood dyscrasias and high rate of thrombotic thrombocytopenic purpura (TTP), it is generally reserved for patients who are intolerant/resistant or allergic to aspirin. Clopidogrel has better safety and tolerability profile, esp. lower incidence of TTP and neutropenia. Clopidogrel also requires hepatic cytochrome P450 to release active metabolite, which binds irreversibly through covalent modification to the P2Y12 receptor such that recovery of platelet function is precluded.[7,11] The drug is equivalent to aspirin in the secondary prevention of stroke and its combination with aspirin appears to be as effective as ticlopidine with aspirin.[12] It is FDA approved for use in stroke, MI, established peripheral arterial disease or ACS. Evidence suggests that there is considerable interindividual variability in response to clopidogrel, as measured by platelet aggregation and activation tests, with 5-10% of patients not responding to its effects, and as many as 25% being only partially responsive to the drug.[13]

GPIIb/IIIa inhibitors

GpIIb/IIIa is a platelet surface integrin which is a dimeric receptor for fibrinogen and vWF, which anchor platelets to foreign surfaces and each other, therefore causing their aggregation.[2,4,5] Hence, inhibitors of this receptor are potent anti-platelet agents e.g., abciximab, tirofiban, and eptifibatide. All these drugs are parenteral preparations. Various pharmacodynamic studies on these drugs demonstrated their antiaggregatory effect to the extent of >80%. The first GPIIb/IIIa receptor antagonist used in clinical settings was abciximab. Abciximab is the Fab fragment of a humanized monoclonal antibody directed against GPIIb/IIIa receptor. It also binds to vitronectin receptor on platelets, smooth muscle cells and vascular endothelial cells. The effect lasts for 18-24 hr, even after the infusion has been stopped. However, use of abciximab is laden with problems of high cost, immunogenicity & irreversibility of the effect.[7,14] Hence, micro molecular GPIIb/IIIa receptor antagonists (e.g., eptifibatide and tirofiban) were developed. Eptifibatide is a cyclic peptide inhibitor of fibrinogen binding site of GPIIb/IIIa receptor. The benefit seen with it is a bit less than abciximab because former doesn't additionally bind to vitronectin receptor. It's duration of action is relatively short, i.e., platelet action restored within 6-12 hr after stopping the infusion.[1,7] Tirofiban is a non-peptide, has a short duration of action is efficacious in non-Q-wave MI with unstable angina, with action similar to eptifibatide. It also doesn't inhibit vitronectin receptors. Both eptifibatide and tirofiban have small molecular weights, therefore are not able to induce an immune response.[7,15] Large-scale clinical trials have demonstrated their clinical effects and safety in ACS. They are used in adjunctive therapy during percutaneous coronary intervention (PCI). However, these drugs are reported to be associated with bleeding risks.[15]

Dipyridamole inhibits phosphodiesterase (PDE) enzyme and primarily has a vasodilator action with little or no benefit as an antithrombotic agent.[7]

LIMITATIONS OF THE CURRENT ANTI-PLATELET DRUGS

Aspirin

Approximately 10-20% of aspirin-treated patients experience a recurrent vascular event within 5 years. This high risk of recurrence has been attributed, in part, to the inability of aspirin to inhibit platelet aggregation in certain patients – so-called aspirin "variability of response" – which is estimated to occur in 5-60% of aspirin-treated patients. The mechanisms of variability in response are not yet fully elucidated, but are considered to be multifactorial, like patient non-compliance, failure of physicians to prescribe aspirin appropriately, drug-drug interactions, cyclo-oxygenase-1 polymorphisms, etc. Another important limitation of aspirin, even at low doses, is the increased risk of gastrointestinal adverse effects, including bleeding.[13]

P2Y12 Receptor antagonists

Both are irreversible anti-platelet agents. Ticlopidine is associated with neutropenia and TTP hence not preferred much.[6] Since clopidogrel requires hepatic cytochrome P450 metabolism to release its active metabolite, there is considerable inter individual variability in response to clopidogrel, with 5-10% of patients not responding to its effects, and as many as 25% being only partially responsive to the drug. As with aspirin, the mechanisms underlying variability in response for clopidogrel are multifactorial such as inadequate dosing, non-compliance, gene polymorphisms of the CYP3A4 system, polymorphisms of the P2Y12 receptor, etc. Patient-tailored treatment has been proposed to solve the problem of non-responders.[3] Further limitations include a relatively modest inhibition of the *ex vivo* platelet aggregation response to ADP, and suboptimal onset of action.[13]

GPIIb/IIIa Inhibitors

They all are parenteral preparations. No drug in this class is available for oral use. Abciximab is not much favored because of its high immunogenicity, irreversibility of action and high cost. Tirofiban and eptifibatide have smaller molecular size, thereby getting rid of the problem of immunogenicity, though at the cost of efficacy, as due to their smaller sizes they have relatively lower affinity at the GPIIb/IIIa receptor. Furthermore, adverse events related to thrombosis or bleeding have still been reported.[7,15]

Hence, all these limitations point toward the need of developing newer targets and agents in anti-platelet therapy.

POTENTIAL TARGETS FOR THE NEWER ANTI-PLATELET DRUGS

Based on the signaling pathways involved in platelet activation, there are four possible targets (Figure 3). Based upon these, a whole pandemonium of novel agents are being evolved, with one of them as a basic mechanism of action[2] (Figure 4).

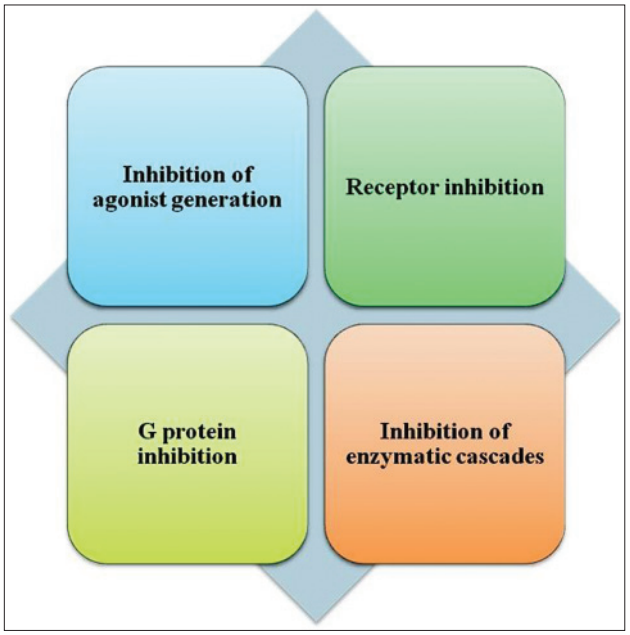

Figure 3: The four possible targets for the anti-platelet action.

NEWER ANTI-PLATELET DRUGS

New P2Y12 antagonists

They can be classified into thienopyridine derivatives and non-thienopyridine derivatives. Thienopyridine derivatives include prasugrel. The non-thienopyridine groups consist of ticagrelor, cangrelor, elinogrel and other numbered compounds. The main difference in the above two groups is reversibility of action, with the non-theinopyridine group being reversible.[13]

Prasugrel

Prasugrel principally differs from the other irreversible thienopyridine P2Y12 receptor antagonists by having an ester group close to the reactive thiol group and a fluorine atom replacing the chlorine atom. Like clopidogrel, prasugrel is a prodrug that requires hepatic metabolism by enzyme CYP2C19 to form its active metabolite which irreversibly inhibits the P2Y12 receptor.[3,13] However, the active metabolite of prasugrel is generated much faster and at higher concentrations compared with clopidogrel, resulting in improved platelet aggregation inhibition and fewer non-responders.[13] The presence of CYP2C19 alleles with less function does not have a clinical impact in prasugrel-treated patients. The degree of inhibition of platelet aggregation obtained with prasugrel within 30 minutes is similar to the peak effect of clopidogrel 6 hrs after administration. However, this benefit was accompanied by a higher incidence of major and fatal bleeding complications. Prasugrel does overcome few limitations of clopidogrel and is therefore preferred in patients undergoing PCI or primary angioplasty, diabetic patients or patients at high risk of thrombosis. However, its irreversibility and the increased hemorrhagic risk remain a problem.[3]

Ticagrelor

Ticagrelor is an oral, reversible, non-thienopyridine, direct-acting inhibitor of the ADP receptor P2Y12. Platelet inhibition is more rapidly and uniformly effective with ticagrelor, and results in less variability compared to clopidogrel. The anti-platelet effect of ticagrelor is independent of CYP2C19 genotype.[16] The advantage of ticagrelor over prasugrel is that it has been tested in the whole spectrum of ACS and there are no "non-responders." The most commonly reported adverse effect with ticagrelor is dyspnoea.[3] Ticagrelor has been evaluated in the study of platelet inhibition and patient outcomes (PLATO) that randomized 18,624 patients with moderate-to-high risk ACS to ticagrelor (180 mg loading dose, 90 mg twice-daily thereafter) or clopidogrel (300-600 mg loading dose, 75 mg daily thereafter). Importantly, patients entered the study regardless of the treatment strategy (invasive or conservative). The primary endpoint, a composite of death from vascular causes, MI or stroke, occurred in 9.8% of patients receiving ticagrelor and in 11.7% of the patients treated with clopidogrel. The rates of death from any cause were also reduced with ticagrelor. No significant difference in the rate of PLATO-defined major bleeding was found in either group, although there was a higher incidence of TIMI major non-CABG-related bleeding in patients who received ticagrelor. Other side-effects were dyspnea and ventricular pauses. However, discontinuation of treatment because of dyspnea occurred in 0.9% of patients in the ticagrelor group and no significant increase was observed for pacemaker implantation.[17] It has been FDA approved in July 2011.

Cangrelor

It is reversible, non-thienopyridine P2Y12 receptor antagonist. It is available only as an intravenous preparation. It has a fast onset of action, as it doesn't need to be metabolized for its effect to appear.[15] In a study comparing cangrelor with abciximab in PCI patients, cangrelor had an almost similar incidence of bleeding complications and recurrent ischemic events, as that of abciximab. At present, there is no evidence to prove its superiority over currently used intravenous anti-platelet agents (e.g., abciximab, etc.) in

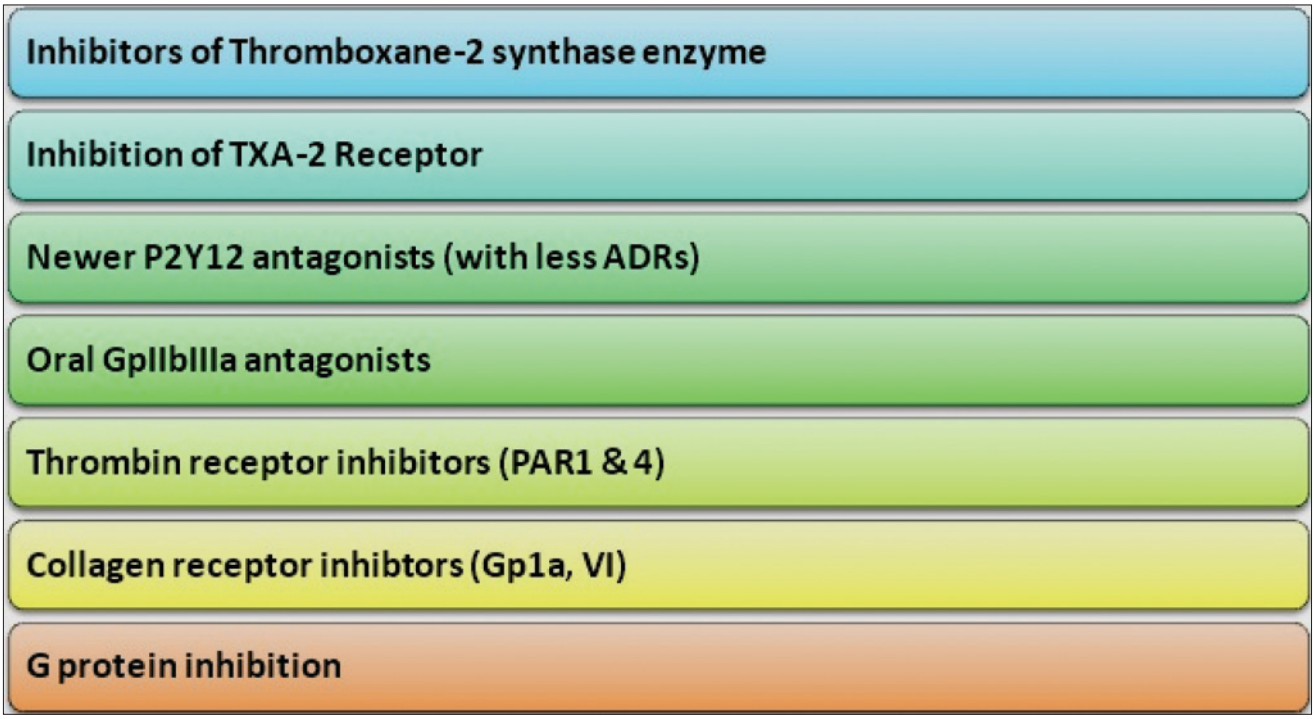

Figure 4: The mechanism of actions of newer anti-platelet agents.

preventing thrombotic events in patients undergoing PCI. The therapeutic potential of cangrelor is therefore not certain.[18] It has a plasma half-life of 3-6 min and is immediately active after infusion: Platelet function normalizes within 30-60 min after discontinuation. Hence, cangrelor is an excellent drug for patients who require a rapid and profound but reversible platelet inhibition.[3]

Elinogrel

Elinogrel is a direct-acting, reversible P2Y12 inhibitor, administered both intravenously and orally. Two pharmacologic properties make it an interesting anti-platelet agent. It is the only P2Y12 receptor inhibitor available in oral and intravenous formulation and both formulations are pharmacologically identical. Studies in healthy volunteers showed great platelet inhibition without clinically relevant adverse effects.[3] In a randomized phase IIA trial, safety and efficacy of Elinogrel in patients with STEMI (ERASE-MI) and on adjunctive anti-platelet therapy showed its efficacy in preventing the thrombotic events with infrequent bleeding events.[19]

Thrombin receptor inhibitors

Thrombin is the most potent platelet agonist and plays a critical role in the development of arterial thrombosis. There are four types of receptors for thrombin and they are protease activated receptors (PAR) 1, 2, 3, & 4. Human platelets express dual thrombin receptors, PAR1 & 4, but there are still no therapeutic strategies that effectively target both receptor subtypes.[20] These receptors are a category of G-protein-coupled receptors implicated in a range of cellular responses such as hemostasis and thrombosis, and inflammation. The ADP and TXA2 mediated platelet activation plays a role in both normal physiological hemostasis and pathological thrombosis. The combined use of aspirin and clopidogrel well inhibits thrombosis but lead to hemorrhagic complications. PAR1-mediated platelet activation predominantly plays a role in pathological thrombosis and doesn't affect the normal physiological process of hemostasis. Hence, PAR1 antagonists decrease the incidence of hemorrhagic complications. The oral PAR1 antagonists, which are currently under the ongoing research, are vorapaxar (SCH 530348) and atopaxar (E-5555).[15]

Vorapaxar

Vorapaxar is a non-peptide competitive PAR1 thrombin receptor antagonist with a high affinity and low molecular weight, blocking thrombin-mediated platelet activation without interfering with thrombin-mediated cleavage of fibrinogen. It has a rapid onset, with great inhibition of platelet aggregation within 1-2 hrs, according to the loading dose. Bleeding time or coagulation time did not increase in preclinical and early clinical studies.[3] In the TRA-PCI study, a phase II trial, 573 patients with ACS who underwent PCI were randomized to vorapaxar (loading dose 10, 20 or 40 mg) or placebo in addition to aspirin and clopidogrel. At 60 days, maintenance doses of 0.5, 1, or 2.5 mg/day were continued. It was not associated with an increase in TIMI major plus minor bleeding versus placebo.[21] Another phase III TRACER study which was to be done on patients with ACS was stopped

early because of bleeding problems. In another phase III trial, TRA 2P-TIMI it was seen that when vorapaxar was given as an add on therapy to standard therapy, there was a significant reduction in secondary cardiovascular events but with an accompanying significant increase in moderate and severe bleeding compared to that of placebo group, and the incidence of intracranial hemorrhages was double in vorapaxar group.[22]

Atopaxar

It is also a powerful oral PAR1 antagonist. It inhibits thrombin mediated aggregation of platelets, without any concomitant increase in bleeding risk, as indicated by the preclinical studies. Furthermore, the fact that it selectively blocks the thrombin receptors present only at platelet surface enables this drug to be used as a potential agent in thrombotic diseases.[1]

In a study comparing the PAR1 antagonists, PAR4 antagonists, and their combination, it was seen that effective inhibition of thrombin-induced platelet aggregation can be only achieved when both the thrombin receptors are inhibited. This has led to research on the role of potential combination therapy of PAR antagonists in thrombotic disorders.[23]

Thromboxane A2 receptor antagonists

It has been already shown in previous studies that TXA2 receptor antagonists have been useful in the management of certain cardiovascular diseases. Recent reports suggest their usefulness in ability to restrict the vascular inflammation in atherosclerotic vessels. Terutroban sodium is a new and highly selective TXA2 receptor antagonist with a long duration of anti-platelet action.[2] In a porcine model of stent induced thrombosis, it was found that the blockade of TXA2 receptor by terutroban sodium provided a fast and potent anti-platelet effect blocking both ADP and collagen induced platelet activation and aggregation. In addition, it was associated with lower bleeding risk.[2,11]

Collagen receptor antagonists

Collagen has been known since a long time to be the main motivator of platelet adhesion, aggregation, and activation, making it a formidable target for the development of new anti-platelet drugs. Collagen receptors offer attractive alternatives for target to inhibit the early stage of platelet activation. Collagen acts on three major receptors, α2β1 integrin (primarily responsible for platelet adhesion to collagen), GPVI (major signaling receptor for collagen, mediates platelet adhesion) & GPIb-V-IX (which acts as a collagen receptor indirectly via vWF). Several experimental studies suggest that these three are interesting targets, for the development of new anti-platelet agents.[15] Platelet adhesion to the exposed sub-endothelial tissue in damaged blood vessel is the first step, leading to arterial thrombosis. vWF forms a bridge between the exposed collagen and interact with the platelets, causing their adhesion to take place to the sub endothelium in the damaged blood vessel wall.[5,6] Therefore, the monoclonal antibodies which target this collagen-vWF-GPIb interaction or which can inhibit another receptor of collagen, i.e., GPVI are being developed for their use in inhibition of platelet aggregation. GPVI is the most attractive target as it was seen that either its inhibition or absence offered huge protection from the development and progression of arterial thrombosis, with no major bleeding in mice. Anti-GPVI antibodies, e.g., JAQ1, have a significant antithrombotic effect *in vivo*.[2]

GPVI antagonists

PR-15 (revacept) is a soluble, dimeric GPVI-Fc that adheres to the exposed collagen in endothelial lesions, thereby preventing the binding of collagen to platelet GPVI receptors. The collagen induced platelet activation and adhesion is greatly reduced with revacept. Similarly, a study in mice having vascular injury, the infusion of revacept almost completely inhibited the platelet arrest and aggregation.[1] A phase I trial of revacept (PR-15) showed it to be safe and well tolerated.[24] DZ-697b is an orally active collagen and ristocetin inhibitor. Phase I trial on it demonstrated it to be safe and efficacious, along with the benefit of shortening of bleeding time, in comparison with clopidogrel.[25] It is currently under clinical investigation.[1]

GPIb receptor antagonists

The drugs that inhibit GPIb receptor are still under investigation and include h6B4-Fab, GPG-290, and SZ2. The 6B4-Fab is a murine monoclonal antibody, derived from the humanized Fab portion of protein that targets GPIbα and thereby neutralizes the binding site of the vWF. It competes with vWF for binding to the receptor GP1bα and inhibits platelet adhesion and aggregation. Moreover, preliminary study done in baboons showed that though, there was no effect on either the platelet count or the bleeding time *in vivo*, but it did effectively inhibit the ristocetin induced platelet aggregation, depending both on time and dose. A chimeric antibody, GPG-290, derived from Chinese hamster ovary cell was effective in preventing the thrombotic event, though it did prolong the bleeding, when compared to clopidogrel. SZ2 is another monoclonal antibody which targets GPIbα, and studies have shown its effectiveness *in vitro*. However, the efficacy of this drug *in vivo* still needs to be demonstrated.[1,15] In recent times, an anopheline anti-platelet protein, AAPP, isolated from the saliva of anopheles stephensi mosquito was found to have a strong and a specific inhibitory effect against collagen induced platelet aggregation. AAPP directly binds to collagen through receptors GPVI and α2β1 integrin, thereby not allowing platelets to adhere to collagen and hence has anti-platelet activity.[2]

vWF antagonists

AJW200 (IgG4 humanized monoclonal antibody to vWF) specifically inhibited platelet aggregation induced under the high-shear-stress conditions *in vitro*.[26] ARC1779 is an aptamer-based antagonist. It has a high affinity to vWF (espA1-domain and inhibits vWF-dependent platelet aggregation.[27] It was shown to prevent platelet aggregation in a phase II trial in patients with TTP, where it remarkably increased the platelet counts. This drug is currently under clinical investigation.[28] Other drugs in this group, under development and investigation are ARC15105, ALX-0081, ALX-0681, & 82D6 A3. ARC15105 is an aptamer which is chemically more advanced and has a higher affinity to vWF. However, in the *ex vivo* trials showed that it is a less specific vWF induced platelet aggregation inhibitor.[29] The preclinical and clinical trials have shown that ALX-0081, a bivalent humanized antibody, is efficacious and safe inhibitor of vWF-mediated platelet aggregation over wide range of doses when administered in combination with aspirin, heparin, and clopidogrel. It is under phase II trials in PCI patients. Further trials will need to follow to verify their efficacy, safety, and tolerability.[1]

Oral GPIIb/IIIa inhibitors

The oral GPIIb/IIIa antagonists have failed to demonstrate their efficacy and safety in various trials, and were associated with significantly increased bleeding risk and mortality in patients with ACS. Therefore, more and more research and testing needs to be done to demonstrate their efficacy as anti-platelet agents in actual patients.[30]

Platelet Gq antagonists

It was proposed that inhibitor of Gq signaling pathway of platelet adhesion will inhibit platelet aggregation by interfering with the GDP-GTP exchange pathway and blocking P2Y1 receptor mediated intracellular calcium mobilization.[2] YM-254890 was seen to inhibit this Gq signaling and is supposed to possess the potential to be used as an anti-platelet drug.[31] However, Gq specific approach is required as inhibition of both Gq and G11 will lead to deleterious effects on multiple organs.[32,33]

PDE inhibitors

The agents that inhibit the platelet aggregation through blockade of platelet membrane receptors have been discussed above. Another class of drugs, which interfere with the intracellular signaling of the platelets, will also exhibit the anti-platelet effect. The major role in intracellular signaling in platelets is played by cAMP and cyclic guanosine 3'-5-monophosphate (cGMP) which are basically second messengers. PDE enzyme is responsible for the hydrolysis of both cAMP and cGMP, thereby indirectly regulating the platelet function. Platelets express three PDE isoenzymes, PDE 2, 3, and 5. Inhibition of either of these PDE enzymes by an agent will confer it with strong anti-platelet action.[15]

Cilostazol

It is an anti-platelet agent, which has been primarily used in the patients of peripheral ischemia, e.g., intermittent claudication. It selectively inhibits cAMP-PDE-3 thereby dilating the blood vessels and blocking the platelet aggregation induced by ADP, collagen and arachidonic acid. As compared to aspirin, cilostazol is reversible anti-platelet drug which is capable of preventing both primary and secondary aggregation. It is metabolized in the liver and excreted by the kidney. All these qualities make cilostazol an effective anti-platelet drug, which has good tolerability and high safety. It is proposed that it may be a better drug than aspirin when it comes to efficacy and safety, in the patients with ischemic stroke.[1] At present, in the patients with ischemic stroke, combining aspirin and cilostazol for better anti-platelet effects seems a pretty good option.[34]

Thromboxane A2 synthase inhibitors

Thromboxane A2 is a potent mediator of platelet aggregation & vasoconstriction. It plays an important role in thrombosis and thrombotic disorders, atherosclerosis, Reynaud's phenomenon etc.[4,5] Thus, TXA2 is a therapeutic target for the anti-platelet activity. Either the drug which blocks its synthesis or its receptor will have an anti-platelet action. Two enzymes are involved in the synthesis of TXA2 from arachidonic acid and they are COX-1 and TXA2 synthase enzyme. Aspirin which is the gold standard drug in anti-thrombotic armamentarium, blocks COX-1 activity. The drugs which inhibit TXA2 synthase are under development and many experimental studies have shown their anti-platelet activity.[35,36] E.g., picotamide has activity both as a thromboxane synthase inhibitor and as a thromboxane receptor antagonist. It was shown to be significantly more effective when compared to aspirin in reducing the mortality associated with peripheral arterial disease in type 2 diabetes mellitus patients, in a study.[37] Dazoxiben is another thromboxane synthase inhibitor which is orally active. It was tested in patients with Reynaud's syndrome and it didn't prove beneficial.[38]

CURRENT STATUS OF ANTI-PLATELET THERAPY

It is by now, clearly seen and understood that the current anti-platelet therapies are generally based on specific signaling pathways in platelet activation, involving single agents acting on single targets and are mainly irreversible. There still lie a bundle of unresolved issues for both the marketed and experimental anti-platelet agents, regarding their efficacy and safety, their optimal dosage, administration requirements, combination therapy, clinical evaluation, cost-effectiveness, and the resistance phenomena.[2] With the daily increase in

both incidence and the associated morbidity and mortality of thrombotic disorders, newer drugs which have anti-thrombotic activity are being researched and investigated adequately for further development.[39,40] The use of combination of anti-platelet drugs having different mechanisms of action might prove to be beneficial in treatment of thrombotic disorders. The ongoing research on platelet receptors and platelet functions might pave the way to the discovery of several newer anti-platelet agents. Several assays for assessing the platelet activity and tests for analyzing the pharmacokinetics of the anti-platelet agent, that can collectively help toward individualizing and optimizing anti-platelet therapy might find their use clinically. However, more studies are needed. There is a hope however; that newer agents with more selectivity and specificity as anti-platelet agents will be developed, which have a very good efficacy profile with minimum bleeding risks.[15]

CRITERIA OF AN IDEAL ANTI-PLATELET DRUG

An ideal anti-platelet agent should specifically block thrombogenic platelet dependent mechanisms in vascular diseases without interfering with normal platelet function which provided the normal hemostasis and wound healing. Furthermore, these drugs should be free from any major adverse effects. None of the currently developed drugs meet these criteria at present. The current most widely used anti-platelet drugs (e.g., aspirin, ADP receptor antagonists, GPIIb/IIIa antagonists and PDE inhibitors) are relatively well tolerated by patients being treated for ischemic diseases. However, the limited efficacy of these drugs in the setting of arterial thrombosis, their unfavorable adverse effect profiles, cost-to-benefit aspirin issues and the "resistance" phenomenon give rise to the need for developing newer agents which have better anti-platelet efficacy and tolerability.[2]

FUTURE DIRECTIONS

For the future development of a "perfect" anti-platelet drug, we need to bring following points into consideration:

1. Shifting our aim from single target toward multiple targets
2. Putting an emphasis on combination therapy, rather than single drug therapy
3. Discovering the anti-platelet activity in already approved and marketed drugs, used for some other indications
4. Aiming for reversibility in action.

Hence, the future drugs should be able to block the platelet activation at multiple steps and should have reversible action, with minimal associated bleeding risk. Furthermore, it is currently being emphasized that combining two anti-platelets with different mechanism of actions is beneficial compared to the single drug therapy alone.[2,15] Also, since the platelet activation is found to be affected by multiple factors e.g., certain pathological states like diabetes, hyperlipidemia, hypertension, etc., drugs that have an activity against these factors have been seen to have indirect platelet inhibitory effects, e.g., ACE inhibitors, HMG CoA reductase inhibitors, etc. Finally, many herbs used in traditional Chinese medicine have been reported to have anti-platelet activity in humans. These herbs have been widely used since ages to treat thrombotic disorders and ischemic diseases in traditional Chinese medicine. Since, they contain multiple active ingredients, their mechanism of action remains unclear. Furthermore, safety and efficacy of these herbs still need to be tested in properly conducted clinical trials. The careful consideration and needful implementation of all these different above mentioned strategies, regarding the development of newer anti-platelet drugs might eventually change the scenario of outcome in cardiovascular diseases by markedly decreasing the platelet activation and aggregation with minimal bleeding risk. Apart from the ongoing research for the newer anti-platelet drugs, it is increasingly becoming clear that platelet activation is a markedly complex process with such an intricate web of intracellular signaling which at present might be beyond understanding. Hence, there is a dire need to unravel new frontiers, primarily based on this complex intracellular signaling process of platelet activation to discover novel compounds which are very specific and with less off target effects. In conclusion, the correct choice of anti-platelet therapy should be based on the patient's thrombotic and bleeding risks.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

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doi: 10.5455/2319-2003.ijbcp20140402
Cite this article as: Ahluwalia K, Bhanwra S. Antiplatelet therapy: present status and its future directions. Int J Basic Clin Pharmacol 2014;3:260-8.