

Research Paper

Platelets and Arterial Thrombosis: Evolving Role of Platelet GPVI as an Antithrombotic Drug Target

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Acute coronary syndrome is one of the leading causes of mortality and morbidity worldwide. Although the recent advancements in existing anti-platelet therapeutics have significantly reduced the burden of cardiovascular deaths but owing to their global impact on primary haemostasis, their clinical benefits are often limited by bleeding risk. Recent developments in the understanding of platelet biology have placed platelet-collagen interaction at the critical position in the sequence of events during arterial thrombosis. Thus there is considerable interest in developing novel therapeutic strategies against platelet collagen receptor glycoprotein VI (GPVI) because not only its selective blockade or deficiency holds promise for the reduced risk of bleeding complications as evidenced in mice and humans, but also the exclusive expression of GPVI on platelets and megakaryocytes offers to prevent the adverse off-target effects of antithrombotic drugs. The present review summarizes recent developments in understanding of platelet functions during arterial thrombosis and structural and functional attributes of platelet GPVI to identify it as a novel antithrombotic drug target. Moreover, it also emphasises on the development of GPVI antagonists using various experimental approaches as well as their prospective use in cardiovascular disorders.

Key Words: Thrombosis; Platelets; Collagen; GPVI; Anti-Platelet Drugs

Introduction

Cardiovascular disorders (CVDs) are a leading cause of death globally, accounting for ~17.3 million deaths per year that have been projected to grow further to >23.6 million by 2030 (Laslett *et al.*, 2012; Smith *et al.*, 2012). Earlier recognized as a disease of industrialised countries, the recent World Health Organization statistics have shown that ~80% of the world's deaths from CVDs affect the younger population in low and middle-income countries, where the socio-economic resources to address them are most limited (Smith *et al.*, 2012). India is experiencing a similar epidemiological health transition characterised by a rapid decline in nutritional and communicable diseases, along with

an alarming rise in CVDs, mainly identified as coronary heart disease and cerebral stroke.

The development of a clot or a thrombus in the coronary or cerebral circulation causes acute myocardial infarction or ischemic stroke respectively. Platelets have a central role in arterial thrombosis as they adhere to the sub-endothelial matrix after endothelial damage due to a ruptured atherosclerotic plaque, and then aggregate with each other to form a prothrombotic surface that promotes clot formation and subsequent vascular occlusion. Despite extensive efforts over the last four decades in the discovery and development of more effective antithrombotic drugs, the effect of existing therapies on mortality rates has remained disappointingly small. The current

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therapies suffer from major limitations like inadequate efficacy against platelet activation, blockade of only single platelet activation mechanism leaving other platelet functions intact, slow onset of action, variability in inter-individual drug response with non-responsiveness of some patients, inconvenient intra-venous route of administration and the adverse bleeding events. This situation has become more challenging with the alarming rise in the incidence of obesity, diabetes and the metabolic syndrome (Bhatt, 2008). Thus, there is a need for the development of more effective approaches to combat this global cardiovascular epidemic.

Hemostasis and Thrombosis: The Central Role of Platelets

The term hemostasis means prevention of blood loss. Whenever a vessel is severed or ruptured, hemostasis is achieved by mechanisms involving: (1) vascular constriction, (2) formation of a platelet plug, (3) formation of a blood clot as a result of blood coagulation, and (4) eventual growth of fibrous tissue into the blood clot to seal the tear in the vessel permanently. Arterial thrombosis is primarily an exaggerated hemostatic response at sites of vascular injury. Giulio Bizzozero, the Italian physician who first defined the role of platelets in hemostasis in 1881, quickly recognised their central contribution to the development of thrombosis. The primary trigger for arterial thrombosis is the rupture of an atherosclerotic plaque, which develops through the accumulation of lipid deposits and lipid-laden macrophages (foam cells) in the artery wall. When an atherosclerotic plaque ruptures, platelets are rapidly recruited to the site through the interaction of specific platelet cell-surface receptors with collagen and von Willebrand (vWF) factor. After this adhesion to the vessel wall, platelet aggregation soon follows which eventually results in rapid growth of thrombus (Goldschmidt, 2002). Activated platelets then release the contents of granules, which further promote platelet recruitment, adhesion, aggregation and activation. Platelets preserve vascular integrity and thereby prevent haemorrhage after injury. However, vascular damage, such as rupture of an atherosclerotic plaque, results in a platelet-dependent

thrombus, which may lead to vascular occlusion with resultant hypoxia and infarction of distal tissues. Thrombotic occlusion of a coronary artery results in acute myocardial infarction and thrombotic occlusion of a cerebral artery results in acute ischaemic stroke (Goldschmidt, 2002).

Platelet Functions: Adhesion, Aggregation and Secretion

Endothelial cells lining the arterial wall continuously release prostacyclin and nitric oxide (NO), thereby keeping the circulating platelets in an inactive state. They also express CD39 on their surface, which converts adenosine diphosphate (ADP), a potent inducer of platelet activation, into adenosine monophosphate (AMP) thereby inhibiting platelet activity. At the site of vascular injury, the platelets adhere to the exposed subendothelial matrix through rapid interactions between collagen, vWF and fibronectin and their receptors on the platelets, integrin $\alpha_2\beta_1$, GPVI, GP Ib-IX-V and integrin $\alpha_5\beta_1$, respectively.

Adhesion over collagen causes platelets to change into an active conformation. Activated platelets secrete ADP, platelet-derived growth factor, and fibrinogen from their storage granules and thromboxane A_2 (TxA_2), produced by immediate biosynthesis. ADP and TxA_2 cause circulating platelets to change shape and become activated. Glycoprotein IIb/IIIa receptors on the surface of activated platelets bind fibrinogen, leading to the formation of fibrinogen bridges between the platelets, resulting in platelet aggregation, which, along with the simultaneous formation of a fibrin mesh, leads to the formation of a platelet thrombus followed by clot retraction that leads to the formation of a stable thrombus. Platelet adhesion to fibrillar collagen also triggers the procoagulant response. In an advanced stage of activation, platelets stimulate blood coagulation by providing a surface at which the coagulation factors are activated to generate thrombin. In addition to providing the procoagulant surface and binding sites for several coagulation factors, platelets contribute to coagulation activity by releasing several substances, such as FV, FXIII,

fibrinogen, vWF, and protein S which play pivotal role in hemostasis.

Anti-Platelet Therapy: Benefits and Pitfalls

Anti-platelet drugs are used for both prevention and acute treatment of arterial thrombosis. These drugs target the activation and the aggregation of platelets and prove highly beneficial in this setting. Existing and investigational anti-platelet drugs target key pathways of platelet activation, which involve surface receptors [e.g. P2Y₁₂, integrin $\alpha_{IIb}\beta_3$, integrin $\alpha_2\beta_1$, PAR1, GPVI, glycoprotein 1b, P-selectin and the thromboxane prostanoid (TP) receptor], signalling proteins [e.g. cyclooxygenase 1 (COX1), phosphodiesterases, phosphoinositide 3-kinase (PI3K), etc.] or endothelial releasates (e.g. nitric oxide) (Goldschmidt Pj 2002).

The presently available anti-platelet agents include aspirin, thienopyridines (clopidogrel), and glycoprotein IIb/IIIa antagonists (Table 1). Aspirin has been shown to reduce atherothrombotic risk in both acute and chronic settings. However, patients on aspirin therapy, particularly those at high risk, may continue to have recurrent thrombotic events. GP IIb/IIIa inhibitors are very potent anti-platelet agents; however, these agents are available only for parenteral

use and have a short duration of action, which impedes their use for long-term protection. Despite the promising rationale behind the use of oral GP IIb/IIIa inhibitors, clinical trials failed to show any benefit of these agents; and a pooled analysis from trials of oral GP IIb/IIIa antagonists showed increased mortality when these agents were given (Chew *et al.*, 2001). The need for alternative anti-platelet treatment strategies led to the evaluation of combination of oral anti-platelet agents inhibiting other platelet-activating pathways.

A multitude of large-scale clinical trials have demonstrated that addition of clopidogrel to aspirin has increased the efficacy in patients undergoing PCI with stent placement [CLASSICS, The Clopidogrel Aspirin Stent International Cooperative Study (Bertrand *et al.*, 2000), CREDO, The Clopidogrel for the Reduction of Events During Observation trial (Steinhubl *et al.*, 2002)], in non-ST segment elevation (non-STE) (Fareed *et al.*, 1999; Gruner *et al.*, 2005), in acute coronary syndrome [CURE, The Clopidogrel in Unstable Angina to Prevent Recurrent Events trial (Yusuf *et al.*, 2001)], and in ST segment elevation myocardial infarction, STEMI [Clopidogrel as Adjunctive Reperfusion Therapy (CLARITY)-Thrombolysis in Myocardial Infarction (TIMI)

Table 1: Existing Anti-Platelet Drugs: Mechanism of action, side effects and limitations

Drug	Mechanism of Action	Side Effects	Limitations
Aspirin	Irreversible acetylation of Ser529 of COX 1	Bleeding Gastrointestinal toxicity: heartburn, indigestion, nausea, vomiting and gastric ulceration	Weak anti-platelet agent
Clopidogrel	The active metabolite irreversibly inhibits P2Y ₁₂ receptors	Bleeding, Rash, Neutropaenia Thrombotic thrombocytopenic purpura (rare)	Patient-to-patient variability in response
Prasugrel	The active metabolite irreversibly inhibits P2Y ₁₂ receptors	Bleeding	• Greater cost than clopidogrel
Abciximab	$\alpha_{IIb}\beta_3$ antagonist	Bleeding	• Thrombocytopenia • Requires IV administration
Eptifibatide	Integrin $\alpha_{IIb}\beta_3$ Antagonist	Bleeding, Thrombocytopenia	Requires IV administration
Cilostazol	Antiplatelet and vasodilatory effects through inhibition of cyclic PDE3	Bleeding, Headache, Diarrhoea, Palpitations, Dizziness, Rash	• Side effects in ~15% of patients led to discontinuation of the drug nucleotide

(Sabatine *et al.*, 2005)]. Thus dual anti-platelet therapy with aspirin plus clopidogrel has become a mainstay of anti-platelet therapy in ACS and percutaneous coronary intervention (Li *et al.*, 2007). Disappointingly, despite the proven benefits of dual anti-platelet therapy, many patients with ACS continue to experience thrombotic events, thereby indicating the strong need for improvement in existing treatments (Maree and Fitzgerald, 2007).

The limitations of existing anti-platelet therapy indicates that additional mechanisms (agonists, receptors and signalling pathways) are required to be regulated during complex platelet functions and also demonstrates functional redundancy between pathways. Therefore, in considering new strategies for the inhibition of platelets, two principal approaches may be used: (1) development of drugs with better efficacies so as to modulate the function of clinically proven and characterised platelet targets or (2) to identify and characterise new targets in platelet pathways. Recent drug development in this area has favoured the former approach (e.g. development of prasugrel), although considerable developments in the understanding of the platelet regulatory machinery in the last two decades, and particularly the detailed characterization of platelet receptors, raise the possibility of finding novel drug targets by latter approaches.

Platelet Collagen Antagonism: A Novel Anti-Platelet Therapeutic Target

Collagen interacts with platelets through direct and indirect mechanisms. At the medium and high shear rates found in arterioles and damaged vascular beds, vWF bridges newly exposed collagen fibres to the GPIb-IX-V complex on the platelet surface. This interaction is facilitated by a fast on-rate of association between vWF and GPIb and is essential for the initial tethering of platelets by collagen at medium to high rates of shear (Savage *et al.*, 1996; Savage *et al.*, 1998). The interaction is opposed by a rapid off-rate dissociation such that platelets translocate (or roll) for several minutes on vWF in the absence of other proteins before forming stable adhesion through activated $\alpha\text{IIb}\beta_3$ (Savage *et al.*, 1996). The situation *in vivo*, however, is very

different, as a number of extracellular matrix proteins work in tandem with vWF to promote stable adhesion, notably collagen.

Besides GPIb-V-IX and $\alpha\text{IIb}\beta_3$ integrin, which interact indirectly with collagen via vWF (Savage *et al.*, 1998), a large number of collagen receptors have been identified on platelets, including most important $\alpha_2\beta_1$ integrin (Santoro, 1986) and glycoprotein VI (GPVI) (Moroï *et al.*, 1989). A current model of platelet adhesion to collagen suggests that the GPIb and vWF interaction mediates initial tethering of platelets at high shear, followed by $\alpha_2\beta_1$ integrin-mediated firm adhesion, which halts platelet translocation and allows collagen interactions with GPVI, finally resulting in platelet activation and thrombus growth (Sixma *et al.*, 1997; Barnes *et al.*, 1998; Santoro, 1999).

GPVI as the Primary Receptor for Platelet Collagen Interaction

GPVI was first identified as a 60 to 65-kDa platelet glycoprotein by 2-D gel electrophoresis more than 20 years ago (Clemetson *et al.*, 1982). The first indication that GPVI may be an important platelet receptor for collagen, however, came from studies on a patient who was presented to clinic with an autoimmune thrombocytopenia caused by autoantibodies to a 65-kDa protein that was present in healthy individuals but absent in the patient (Clemetson *et al.*, 1982). Gel electrophoresis (2-D) demonstrated that the patient antiserum recognised GPVI. Platelets from this patient were unresponsive to collagen, whereas activation by other stimuli was normal. These early studies on the GPVI-deficient patients provided compelling evidence for a key role of the glycoprotein in platelet activation by collagen, but this was not initially recognised because of the multiplicity of other candidates for this role.

It has been accepted for many years that GPVI is essential for platelet activation and is required for adhesion over collagen. It was, therefore, an unexpected finding that GPVI-depleted mouse platelets show virtually no adhesion to collagen under static and flow conditions even though they express normal levels of the major adhesion receptors, GPIb-

V-IX, and $\alpha\text{IIb}\beta_3$ (Nieswandt *et al.*, 2000). In later studies, the role of GPVI was identified to primarily generate intracellular signals that promote integrin activation rather than to serve as an adhesion receptor. Studies in mice have shown that adhesion over collagen under static conditions is maintained in platelets expressing 20-50% of the control level of GPVI, thereby demonstrating GPVI, even in a very low copy number, is sufficient to induce integrin activation. However, under moderate to high shear flow conditions, the GPVI-depleted platelets are unable to adhere to collagen, demonstrating that strong integrin activation is essential for shear-resistant adhesion. Together, these observations have provided compelling evidence that GPVI plays a key role in the process of intra-platelet signalling and activation, which is a prerequisite for platelet adhesion over collagen.

It is evident that platelet regulates the blood clotting process in a multi-factorial complex series of interacting processes. The initial capturing of platelets from the circulation varies with the shear stress experienced at the site of injury and plasma vWF brings about this initial interaction. This is followed by the secure placement of platelet over exposed collagen surface, for which integrin $\alpha_2\beta_1$ is considered to be crucial. Finally, GPVI being the primary signalling collagen receptor expressed on the platelet surface activates platelets to complete the act. However, the exact role of these individual interactions in the capture and activation of platelets remains debatable, and it seems most likely that, once contact is established, all processes proceed immediately. GPVI is central to the signalling process and in up-regulating the affinity of integrins to enhance their adhesive properties.

Platelet GPVI: Structural and Functional Insights

Elucidation of the mechanism of interaction of platelet GPVI with the fibrillar collagen is an essential step towards understanding the complex process of platelet activation in the initial stages of hemostasis and thrombosis (O'Connor *et al.*, 2006). The fact that despite being an immune-type receptor, platelet GPVI recognizes fibrous collagen has provoked further

exploration of its structural characteristics. The atomic structure of GPVI facilitated the identification of potential regions involved in interaction with collagen and these may serve as putative targets for anti-platelet drugs. Moreover, introduction of a number of molecular tools to investigate the GPVI-collagen interaction has further contributed to the existing pool of information: recombinant soluble Ig-like domains of GPVI termed D1D2, recombinant single chain variable domain antibody fragments, selected on D1D2 and a series of synthetic triple-helical peptides that are structurally well defined and are recognized by GPVI (Horii *et al.*, 2006).

To inhibit platelet aggregation, an antagonist needs to inhibit GPVI binding to the minimum recognition motif in collagen. The preliminary studies utilised the collagen related peptide in order to map the primary collagen-binding site on human GPVI. Furthermore, studies using site-directed mutagenesis and antibody V gene phage display technology suggested that the first domain of GPVI makes an important contribution towards collagen binding. This property may be attributed to the presence of a patch of basic residues on its surface, which gets disrupted following mutation of K59. Crystallographic studies of purified GPVI further substantiated the fact that its collagen-binding domain (CBD) is composed of two Ig-like domain (D1 and D2) oriented 90° apart. GPVI has also been shown to differ from other LRC receptors owing to an 11-residue deletion in the sequence of D1 which creates a shallow groove thereby forming a putative collagen-binding site (Horii *et al.*, 2006). Furthermore, the collagen binding domain (CBD) has been proposed to form a back-to-back dimer in the crystal in which two putative collagen-binding grooves are nearly parallel and separated by 5.5 nm; a configuration that matches the orientation and dimension of triple helices within fibrous collagen. Moreover, various single amino acid mutants of human GPVI like R60, R166 and K59 showed reduced binding to collagen in contrast to CRP in which only the mutation of K59 showed reduced binding. Thus, CRP and collagen-binding sites of human GPVI have been proposed to overlap; although the binding sites are chemically different.

Additionally, observation that loss of N-linked glycan at the N92 residue of GPVI has a more profound effect on binding to CRP or type I collagen than to CVX gave further support to the hypothesis that there are 2 distinct but overlapping binding sites (Kunicki *et al.*, 2005). In soluble GPVI chimeric proteins it was observed that collagen binding requires contribution from both domains D1 and D2, although D1 was more efficient in restoring binding to collagen than D2 (Dumont *et al.*, 2006). This finding was in confirmation with the previous crystallographic and mutagenesis data. The classical feature of immunoglobulin receptor family is that the Ig-like loops do not participate in agonist binding. Platelet GPVI was identified to be exceptional since the second ectodomain D2 made an important and specific contribution to GPVI binding to collagen by probably interacting with the D2 domain of other GPVI molecule thereby causing parallel back-to-back GPVI dimerisation.

Relevance of Collagen Antagonism as Drug Target: Past, Present and Future

Platelet GPVI antagonism has been identified to be one of the most promising approaches for clinical intervention of intravascular thrombosis as evidenced from the extensive experimental and clinical studies conducted on the role of platelet GPVI and the regulation of its expression and shedding. The preliminary reports on the effects of GPVI blockade or deficiency in animal models of arterial thrombosis or ischemic stroke have highlighted the therapeutic potential of competitive inhibitors of collagen-GPVI (Massberg *et al.*, 2004; Gruner *et al.*, 2005; Kleinschnitz *et al.*, 2007; Maree and Fitzgerald, 2007; Stoll *et al.*, 2008; Takayama *et al.*, 2008; Bender *et al.*, 2011). The anti-platelet strategies targeting platelet-collagen interaction are considered to be more selective for pathological thrombus formation without compromising haemostasis because the abrogation of GPVI function in human or other animals generally have minimal impact on bleeding time (Massberg *et al.*, 2003; Nieswandt and Watson, 2003; Arthur *et al.*, 2007; Maree and Fitzgerald, 2007; Jung and Moroi, 2008; Takayama *et al.*, 2008; Ungerer *et al.*, 2011). Although more confirmation

on this aspect is still awaited since some individuals with GPVI defects suffer from bleeding disorders, which might depend on other contributing factors or on the type of insult (Arthur *et al.*, 2007). The currently available approaches for targeting platelet-collagen interaction include anti-GPVI antibodies (single chain or humanized forms) (Maree and Fitzgerald, 2007), recombinant soluble dimeric GPVI-Fc fusion protein (Miura *et al.*, 2002; Massberg *et al.*, 2004; Gruner *et al.*, 2005; Bultmann *et al.*, 2006; Jung *et al.*, 2009; Ungerer *et al.*, 2011), insect proteins and synthetic molecules. The soluble dimeric GPVI-Fc fusion protein, when conjugated with a detectable probe, has also been tried for intravascular or *ex vivo* imaging of exposed collagen in lesion prone regions (Bigalke *et al.*, 2011). Furthermore, a recent study identified a novel class of synthetic carboxamide derivatives of N-substituted pyroglutamic acid and higher homologs with selectively substituted cyclic diamines (aminomethyl piperidines) analogues, which exhibited specific inhibition of collagen-induced platelet aggregation without affecting other regulatory and physiologically relevant platelet functions, and thus provided a clinically useful lead to develop new anti-thrombotic agent (Dikshit *et al.*, 2012).

In contrast to a number of high performing anti-platelet drugs, which inhibit all platelets and therefore interfere with haemostasis, compounds that specifically interfere in the collagen-induced activation of platelets have been projected to hold promise for reduced risk of complications. One of the strategies is to identify novel exogenous factors from animal sources that directly interfere with the platelet-collagen interaction at the site of injury. Anopheline antiplatelet protein (AAPP), isolated from the saliva of malaria vector mosquito (*Anopheles stephensi*), was found to block platelet adhesion and activation by directly binding to collagen (Yoshida *et al.*, 2008). Similarly Triplatin-1 and -2, isolated from *Triatoma infestans*, were reported to be the first natural inhibitors of GPVI that selectively inhibited the collagen induced platelet aggregation (Morita *et al.*, 2006). A number of drugs have been developed or are in the process of being developed from animal

sources, for example, desmoteplase (a plasminogen activator) from vampire bat saliva, (Gardell *et al.*, 1989; Witt *et al.*, 1992; Furlan *et al.*, 2006), calin (a plasminogen activator) from *Hirudo medicinalis* leech saliva (Deckmyn *et al.*, 1995; Harsfalvi *et al.*, 1995); and hirudin (an anticoagulant) from *H medicinalis* leech saliva (Fareed *et al.*, 1999; Tardy *et al.*, 2006). Thus, further investigations on the inhibitory mechanisms of these insect proteins might provide new insights into the development of anti-platelet agents that antagonize thrombus formation at the initial phase.

In another approach, Revcept (soluble dimeric GPVI-Fc fusion protein) is directed against collagen in endothelial lesions, which consequently scavenges the main ligand for the activation of crucial platelet receptors. In the pre-clinical animal studies as well as phase I clinical study, it was demonstrated that Revcept dose-dependently inhibited collagen responsiveness and importantly, showed no early signs of aberrant bleeding (Ungerer *et al.*, 2011), which indicated it to be a safe and well-tolerated molecule, that binds to collagen and fibronectin at the atherosclerotic plaque, thereby preventing platelet adhesion and consequent thrombus formation at the site of vascular injury (Fig. 1). This innovative strategy to block the early source of acute vascular complications might prove successful in excluding the disadvantages of existing anti-platelet drugs that primarily rely upon targeting the consequences of platelet activation. However, direct binding to collagen *in vivo* might have alternative undesired consequences. It has been recently shown that under hyperglycaemic conditions, plasma kallikrein binds to collagen and inhibits collagen induced platelet aggregation, thereby enhancing cerebral haematoma expansion in rodents (Bender *et al.*, 2011; Liu *et al.*, 2011). This observation presented the probable safety issues associated with the blockade of GPVI or collagen by any approach and hence should be considered with caution.

Another approach to attenuate platelet collagen responsiveness is to deplete platelet GPVI *in vivo*, by using anti-GPVI monoclonal antibodies or suitable derivatives (Massberg *et al.*, 2003; Takayama *et al.*,

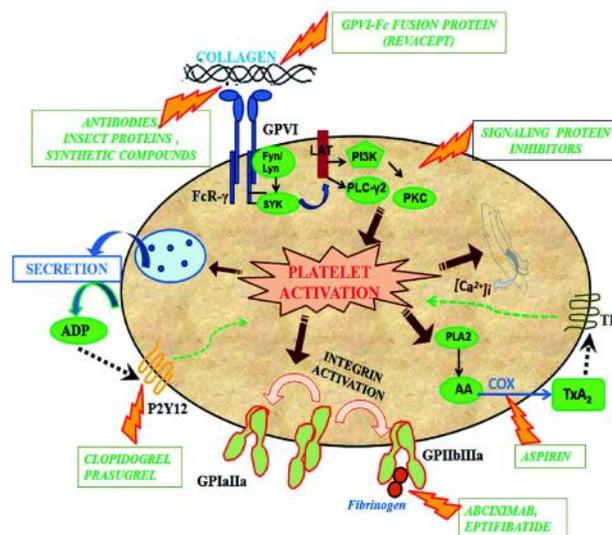


Fig. 1: Platelet GPVI as an antithrombotic drug target. Schematic representation of major adhesion and activation mechanisms responsible for hemostatic and thrombotic functions of platelets. The interaction of platelets with subendothelial matrix following an endothelial injury triggers thrombus formation. Collagen being the major component of the subendothelial matrix induces platelet adhesion and activation. Platelet glycoprotein VI (GPVI) plays a crucial role in platelet response to collagen. It is non-covalently associated with the ITAM-bearing receptor, FcR γ -chain and belongs to the immunoglobulin (Ig) superfamily. The signalling pathway coupled to the GPVI-FcR γ complex involves a cascade of Tyr-phosphorylation by Src-family kinases and the association of the adaptor protein LAT with PI3-kinase, which eventually leads to PLC- γ 2 activation, increase in intracellular calcium concentration and platelet activation (i.e. secretion of granule content, aggregation, procoagulant activity). A regulated cross-talk between GPVI, integrins, and receptors for ADP (P2Y12) and TxA₂ achieves stable platelet adhesion to subendothelial collagen and thrombus formation. GPVI is thus considered to be an attractive target for the development of new antithrombotic drugs. GP, glycoprotein; TxA₂, thromboxane A₂; TP, thromboxane/prostanoid receptors; ADP, Adenosine Diphosphate; P2Y12, purinergic receptors

2008). The peak plasma concentrations, half-life and other pharmacokinetic properties of mF1232 antibody in monkeys enable prolonged inhibition of collagen responsiveness for more than a week, and basically cause cAMP dependent endocytosis of platelet GPVI (Takayama *et al.*, 2008). The anti-GPVI antibodies offer high selectivity towards their target GPVI due to their limited cellular distribution and specificity.

However, apart from prolonged platelet inhibition, the presence of anti-GPVI auto-antibodies in several subjects are believed to cause low platelet counts with a mild bleeding disorder (Moroi *et al.*, 1989; Boylan *et al.*, 2004). Moreover, when such antibodies were administered in combination with existing antithrombotic drug aspirin, hemostasis was severely compromised in mice (Gruner *et al.*, 2004). Moreover, these types of agents are not likely to be orally available, and might not be suitable for long-term anti-platelet clinical use (Fig. 1).

Recently, it was reported that Angiotensin II Type I (AT1) receptor antagonist, Losartan and its active metabolite EXP3179, specifically inhibit collagen induced platelet activation and subsequent thrombus formation via GPVI (Grothusen *et al.*, 2007). The NMR spectroscopy and *in silico* tools further verified that Losartan binds GPVI at the site which overlaps with the putative CRP binding site (Ono *et al.*, 2010). Hence, it was proposed that the losartan binding site in GPVI could be an attractive site for small molecule inhibition of the GPVI-collagen interaction. Moreover, losartan can be utilized as a model chemotype and its key chemical structure is known to bind GPVI which can offer additional possibilities to design molecules with improved activity and specificity as GPVI antagonists (Ono *et al.*, 2010). The solving of the crystal structure of the GPVI ectodomain, the likelihood that GPVI forms a functional homodimer on the platelet surface via an interaction between the ectodomains and identification of the putative residues in the GPVI ectodomain involved in its interaction with collagen, provide further insights and opportunities for screening/developing new small molecule competitive or allosteric inhibitors (Arthur *et al.*, 2005; Herr, 2009; Jung *et al.*, 2009; Kato-Takagaki *et al.*, 2009).

Summary

Aberrant platelet activation plays a crucial role during pathogenesis of arterial thrombosis and therefore, platelet activation and signalling mechanisms represent a major target for pharmacological intervention. During recent years, increasing amounts of experimental and clinical studies have greatly improved our understanding of platelet-collagen interaction that instigates platelet adhesion and activation. After substantial confirmation of their safety and efficacy, inhibitors of collagen-induced platelet activation have been proposed to have therapeutic applications to address major cardiovascular pathologies. Such innovative approaches are needed, considering that a plethora of attempts to implement novel thrombolytics or platelet inhibitors have achieved limited success in the past. Intensive research led to the identification of collagen antagonism as a promising therapeutic target and it is highly encouraging that several new compounds are presently enrolled in late pre-clinical and even clinical studies. The presently available strategies to block platelet-collagen interaction include GPVI depleting antibodies (JAQ1, mF1232), collagen binding fusion protein (Revacept), caffeic acid ester and proteins derived from insects (rAAPP, Triplatin). However, except Revacept, which recently completed its Phase I clinical trial, all other strategies are still under initial stage of development and have yet to be successfully developed and/or clinically deployed. Moreover, except for Losartan (AT1 receptor antagonist) and its active metabolite EXP-3179, there is no substantial report of synthetic small molecule inhibitor targeting platelet GPVI that can be orally administered to the patients. Therefore, the huge potential of platelet GPVI invites innovative and translational studies for the future understanding and development of more specific and efficacious anti-platelet drugs for the treatment of cardiovascular pathologies.

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