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REVIEW

Targeting GPVI as a novel antithrombotic strategy

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Abstract: While platelet activation is essential to maintain blood vessel patency and minimize loss of blood upon injury, untimely or excessive activity can lead to unwanted platelet activation and aggregation. Resultant thrombosis has the potential to block blood vessels, causing myocardial infarction or stroke. To tackle this major cause of mortality, clinical therapies that target platelet responsiveness (antiplatelet therapy) can successfully reduce cardiovascular events, especially in people at higher risk; however, all current antiplatelet therapies carry an increased probability of bleeding. This review will evaluate new and emerging targets for antithrombotics, focusing particularly on platelet glycoprotein VI, as blockade or depletion of this platelet-specific receptor conveys benefits in experimental models of thrombosis and thromboinflammation without causing major bleeding complications.

Keywords: platelet, bleeding, antithrombotic, hemostasis, glycoprotein, vessel, thrombosis

Introduction

The platelet response to vessel injury or infection is essential for normal hemostasis. Platelets also have multifaceted roles in inflammation and immunity.[1] In response to vascular injury, circulating platelets rapidly adhere to exposed subendothelial matrix proteins, such as von Willebrand factor (VWF) and collagen.[2,3] Adherent platelets become activated, spread, and release the contents of storage organelles. These are dense bodies that contain prothrombotic substances, including serotonin and adenosine diphosphate (ADP), membrane glycoproteins P-selectin and CD40 ligand, coagulation proteins, fibrinolytic proteins, growth factors, cytokines, and chemokines.[4] The platelet response is enhanced by signaling pathways initiated through the thromboxane A2 receptor and $P2Y_1$ and $P2Y_{12}$ receptors for ADP, ultimately leading to the activation of the platelet-specific integrin $\alpha IIb\beta 3$ and platelet aggregation, thereby maintaining blood vessel patency and minimizing the loss of blood upon injury.

Myocardial infarction or stroke can result from untimely or excessive platelet activity leading to unwanted platelet activation and aggregation, causing thrombosis. Antiplatelet therapies target the ADP receptor $P2Y_{12}$ (thienopyridine-based inhibitors clopidogrel and prasugrel) or thromboxane generation (cyclooxygenase inhibitor, acetylsalicylic acid) and aim to modulate platelet responsiveness in people at risk of thrombosis.[5] When given together (dual antiplatelet therapy), these treatments significantly reduce the risk of vascular events in high-risk patients.[6,7] Critically, however, the risk of bleeding in patients also increases with the use of antiplatelet therapies.[8,9]

There is a clear need to develop new antiplatelet reagents ideally with high antithrombotic properties but negligible effects on normal hemostasis. Platelet

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glycoprotein (GP)VI represents an attractive new target as an antiplatelet reagent, because it is only expressed on platelets and platelet precursor cells in bone marrow (megakaryocytes), and GPVI blockade has demonstrated efficient antithrombotic potential in the experimental models of thrombosis without enhancing pathological bleeding. Targeting GPVI in the setting of myocardial infarction makes good sense as platelet levels of GPVI are elevated in people who have coronary syndromes.[10–12] More recent data indicate that the blockade of GPVI may also be advantageous for therapies that target inflammatory processes involving platelet function. As several excellent reviews on this topic have been published elsewhere recently,[13–16] we will briefly cover the background rationale for targeting GPVI before highlighting the existing and novel approaches to modulate GPVI function.

GPVI structural and functional features

GPVI is found exclusively on platelets and megakaryocytes and is the predominant platelet receptor for collagen.[17] GPVI has also been shown to bind laminin, an additional extracellular matrix protein.[18] Human GPVI is an approximately 64 kDa type I transmembrane glycoprotein of the immunoreceptor family, with two extracellular immunoglobulin (Ig)-like domains, an extracellular mucin-like domain, followed by a 19-amino acid transmembrane domain, and a cytoplasmic tail of approximately 50 residues that is important for the transmission of ligand-regulated signals.[19] Platelets from healthy donors contain approximately 10,000 copies per platelet of GPVI.[20] Critical residues within the transmembrane domain of GPVI enable this receptor to link with the fragment crystallizable (Fc) γ chain and form a cooperative signaling complex, which utilizes two immunoreceptor tyrosine-based activation motifs (ITAMs) within the cytoplasmic tail portions of dimerized Fcγ chain.[21,22] The cytoplasmic tail of GPVI also contains a proline-rich region that can recruit sarcoma (Src) family kinase members[23] and a calmodulin-binding sequence in the juxtamembrane region.[24] The Ig domains of GPVI[25] are able to bind specific glycine–proline–hydroxyproline motifs within collagen[26] and, in doing so, cluster GPVI on the platelet membrane to enhance the signaling response triggered by binding collagen.[17]

Like many other Ig-like receptor family members,[27–29] GPVI can exist as a dimer on the platelet surface.[30,31] Dimerization is enhanced by ligand binding and by platelet activation.[31,32] In the case of GPVI, dimers can be stabilized via the formation of a disulfide bond between cysteinyl thiol groups on the penultimate residues within the cytoplasmic tails of the adjacent GPVI molecules.[27] Information gleaned from the crystal structure of the ligand-binding Ig-like domains of GPVI also hints that an interaction between adjacent GPVI ectodomains is likely.[33] Such a receptor dimerization step is likely to enhance ligand-induced signaling and to strengthen collagen-binding, as the affinity of dimerized GPVI ectodomains for collagen is significantly greater than for the monomer.[30,34]

An additional feature of GPVI is that the receptor can be downregulated at the platelet membrane.[35] This modulation of receptor levels can be achieved by the cleavage of the ectodomain upon the ligand engagement of GPVI[36] or FcγRIIa[37] by activation of the coagulation cascade[38] or exposure to elevated levels of shear stress.[39] Under certain experimental conditions, GPVI may also be internalized and degraded.[40,41]

Throughout biology, the shedding of the receptor ligand-binding ectodomains is a consistent recurring mechanism that is used to regulate the function of adhesion and signaling receptors.[42–44] This irreversible process occurs within seconds to minutes of the exposure of the platelets to collagen or to pathophysiological levels of shear and may be a mechanism by which ligand- or shear-exposed platelets can reduce levels of functional GPVI at the membrane surface and downregulate activation signals. This process is mediated by members of the a disintegrin and metalloproteinase (ADAM) family of membrane-bound metalloproteinases, predominantly ADAM10 in the human system.[45] In nucleated cells, ADAMs are synthesized and stored as proenzymes within the cytoplasmic vesicles. Upon appropriate stimulation, they are then enzymatically processed to remove the prodomain and brought to the surface of the cell as an active metalloproteinase.[46] This activation process can take 4–16 hours. In platelets, the ADAM proteolytic processing of substrates can be detected within seconds to minutes of platelet activation. There is no evidence that platelets store zymogen forms of ADAMs, implying that the ADAMs proteins are present on the surface of nonactivated platelets (Qiao, Andrews, Arthur, Gardiner; unpublished data, 2012). A peptide with sequence matching residues 228–248 of the extracellular juxtamembrane sequence within GPVI could be proteolyzed by recombinant ADAM10 at position R242–Q243.[45] This site is also presumably present and accessible for enzymatic cleavage on the circulating platelet surface. Cleavage within this region of GPVI results in the liberation of an approximately 55 kDa ectodomain fragment of GPVI and production of an approximately 10 kDa remnant portion that remains associated with the platelet surface. However, unlike other platelet receptors that are constitutively shed from

the platelet surface, the majority of GPVI remains intact on platelets under resting conditions. The proteolytic cleavage of GPVI only occurs upon specific activation of platelets.

Platelet processes mediating thrombosis

Platelet adhesion and activation are mediated by one or more adhesion-signaling receptors on the platelet membrane (Figure 1). Principal players in these adhesive events are the receptors GPIb-IX-V that binds VWF as well as other important vascular proteins, (P-selectin, leukocyte integrin αMβ2, thrombin, high molecular weight kininogen, thrombospondin-1, factor XI, factor XII) and GPVI which binds collagen and also laminin,[18] both of which are exposed within damaged vascular walls.

Importantly, GPIb-IX-V and GPVI engage their respective ligands differentially, according to local blood rheological conditions, with GPVI binding collagen exposed within the blood vessel walls at relatively low physiological shear rates[17] and GPIb-IX-V binding VWF after exposure to high shear rates as found in arterioles and stenotic arteries.[48]

Engagement of either of these receptors leads to the activation of intracellular signaling pathways leading to the upregulation of platelet-specific αIIbβ3 integrin and resulting in enhanced ability of αIIbβ3 to bind the plasma protein fibrinogen.[49,50] Triggering of these signaling pathways also initiates or enhances metalloproteolytic shedding of the extracellular ligand-binding portions of GPVI,[36] and GPIbα and GPV.[45,51]

Interestingly, GPIbα and GPVI are directly and functionally linked on the platelet surface[52] and activate common signaling pathways,[53] further underscoring the extent of cooperation between these adhesion molecules. This differential involvement of GPIb-IX-V and GPVI in the initiation of platelet responses implies a coordinated response by platelets determined at least in part by specific vascular

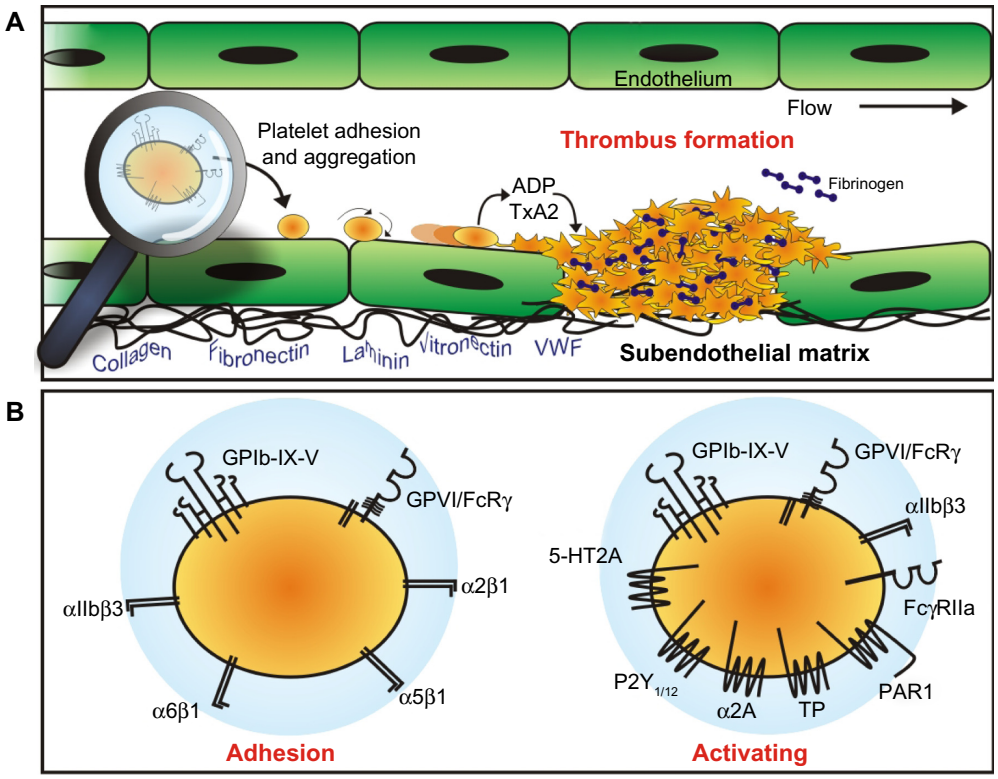

Figure 1 Platelet adhesion and aggregation.

Notes: (**A**) Platelets normally circulate through the vasculature in a nonadhesive state. Upon the detection of an exposed subendothelial matrix, platelets are induced to come into close contact with the vessel wall and roll, then arrest, at the site of vessel injury. The process of adhesion is orchestrated by the platelet adhesion receptors GPVI and GPIb-IX-V. The release of soluble agonists, such as ADP and thromboxane A2 (TxA2) amplify platelet activation. Platelet adhesion and activation, results in the formation of a platelet plug (thrombus). (**B**) Platelet engagement with the blood vessel wall is predominantly mediated by GPVI and GPIb-IX-V; however, the platelet surface possesses receptors that can engage matrix proteins. Additional involvement of these other adhesion proteins, including integrins α2β1, α5β1, and α6β1, which bind collagen, fibronectin, and laminin, respectively, and αIIbβ3 that binds VWF and fibrinogen, among others, help to stabilize the initial attachment and facilitate platelet recruitment and thrombus growth. Platelet activation occurs following agonist binding to GPIb-IX-V and GPVI, integrin αIIbβ3, FcγRIIa, and the G protein-coupled receptors for serotonin (5-HT2A), ADP ($P2Y_{1/12}$), epinephrine (α2A adrenergic receptor), TxA2 (TP), and thrombin (PAR1).

Abbreviations: ADP, adenosine diphosphate; VWF, von Willebrand factor; GP, glycoprotein.

conditions and may provide an opportunity to selectively target a single prothrombotic process from one adhesion receptor while maintaining an adequate response from the other adhesion receptor.

Intracellular molecular signals that result from the engagement of either GPIb-IX-V or GPVI by their ligands may also represent attractive targets for antiplatelet therapeutics (Figure 2). The signaling cascades involve similar events where receptor binding results in the activation of the Src family kinases followed sequentially by spleen tyrosine kinase (Syk), lymphocyte cytosolic protein 2 (also know as SLP-76), phosphatidylinositide (PI) 3-kinase, Bruton's tyrosine kinase (Btk), phospholipase (PL) Cγ2, and protein kinase (PK) C, ultimately resulting in the elevation of the cytosolic Ca^{2+} and αIIbβ3 activation.[54,55] Small differences exist regarding the order of activation of the signaling molecules and some signaling/adaptor molecules. For example, Src and Lyn appear to be recruited to the GPIb-IX-V complex upon platelet activation, whereas Fyn and Lyn are constitutively associated with GPVI.

Additionally, it is reported that GPVI activation induces significantly more inositol phosphate production and PLCγ2 activity relative to GPIb-IX-V activation.[55] Differences regarding the tyrosine phosphorylation patterns downstream of GPIbα and GPVI, where the GPVI agonists induce more rapid tyrosine phosphorylation of platelet proteins relative to GPIbα engagement, are also evident.[56] The engagement of GPVI also produces significant amounts of intracellular reactive oxygen species (ROS), possibly as part of the signaling process[57] and the blockade of the ROS-generating nicotinamide adenine dinucleotide phosphate ($NADP^+$)-oxidase downstream of GPVI engagement, reduces platelet activation and aggregation, and results in the formation of smaller thrombi,[58,59] possibly via the ablation of thromboxane production.[59] ROS-generating machinery is also associated with the cytoplasmic tails of the GPIb-IX-V complex;[60] however, evidence that the engagement of GPIb-IX-V also leads to the generation of ROS has not yet emerged. These small differences in signaling responses between GPVI and GPIb-IX-V, once engaged by their respective ligands, may provide some biological leads for the design of agents that would selectively target one receptor over the other and so provide tools to mediate platelet activation under the differential shear conditions found in specific vascular beds.

GPVI involvement in thrombosis

In vascular lesions, where the endothelium has been denuded/disrupted or there exists plaques, collagen as well as VWF are exposed and form a prothrombotic surface, a potent trigger for the platelet adhesion and aggregation.[2] Platelet engagement with the blood vessel wall is predominantly mediated by GPVI and GPIb-IX-V, which cooperate under a large range of blood shear conditions. Additional involvement of other adhesion proteins – including integrins α2β1[61,62]

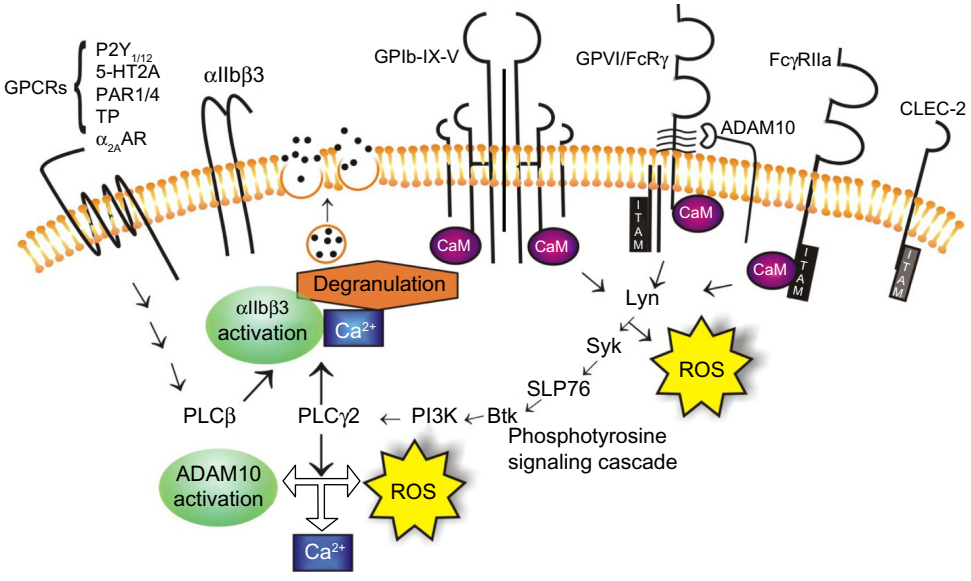

Figure 2 Signaling pathways orchestrate platelet activation and aggregation.
Notes: Engagement of platelet adhesion receptors triggers a phosphotyrosine-signaling cascade that leads to αIIbβ3 activation, ADAM10 activation, ROS production, calcium flux, and degranulation.
Abbreviations: GPCR, G protein-coupled receptor; GP, glycoprotein; ADAM, a disintegrin and metalloproteinase; ROS, reactive oxygen species; Syk, spleen tyrosine kinase; Btk, Bruton's tyrosine kinase; CLEC-2, C-type lectin receptor-2; PLC, phospholipase C; PI3K, phosphatidylinositide 3-kinase; CaM, calmodulin; ITAM, immunoreceptor tyrosine-based activation motif.

and $\alpha 6\beta 1$[63] – helps to stabilize the initial attachment and facilitate platelet recruitment and thrombus growth.

However, the engagement of GPIb-IX-V and GPVI generates a cascade of signaling events that lead to Ca^{2+} mobilization, the rapid release of a battery of soluble agonists that includes ADP and thromboxane, inorganic polyphosphates,[64] microparticles, and thiol oxidoreductase ERp57,[65] and produces a negatively charged phosphatidylserine-expressing platelet surface[66,67] to aid and enhance the generation of active tissue factor.

Consequences of reduction of GPVI function

Table 1 provides a summary of the known data collected from both mouse and human systems evaluating the contribution of GPVI to normal hemostasis and to thrombosis and other responses involving platelets. Platelets with reduced/absent GPVI, as found in people with platelet-targeting autoantibodies[68,69] or genetic mutation,[70–72] display a mild-to-more-severe bleeding diathesis that can include ecchymosis, epistaxis, easy bruising, and prolonged bleeding from mucosal membranes and gums. Experimentally, these platelets display reduced response to collagen and other GPVI agonists by aggregation or flow cytometry-based assays[69,73] and a reduction in thrombus size when exposed to collagen in ex vivo blood flow-based assays.[74]

Similarly, platelets from mice genetically deficient for the GPVI/Fcγ chain[75,76] or treated with anti-GPVI antibodies that cause the removal of GPVI from platelets[76,77] do not respond efficiently or effectively to collagen in assays in vitro. Significantly, however, mice that are genetically deficient in GPVI do not display a prolonged bleeding time, but they do demonstrate a moderate-to-strong protection from thrombosis, depending on the injury model employed.[75,78–81] Clearly, in animal models of thrombosis, the initiating event and the vascular bed being studied in part determine the extent of the contribution and the relative importance of GPVI to platelet activation and thrombus formation (Table 1).

Table 1 Extent of involvement of GPVI in onset of thrombotic and inflammatory disorders

	Type of injury	GPVI involvement
Hemostasis	Minor vascular injury[72,94]	Minor
Venous thrombosis	Low shear, blood stasis, coagulopathy[80,83]	Minor
Arterial thrombosis	High shear, vascular damage[77,114]	Major
Atherothrombosis	Plaque rupture, exposure of thrombogenic material, occlusion[115]	Major
Ischemic stroke	Thromboembolic occlusion[116,117]	Major
Inflammation	Collagen fragments, immune complexes ischemia/reperfusion injury[95–97,118]	Major

Abbreviation: GP, glycoprotein.

Under normal or healthy blood rheological conditions, the contribution of GPVI is only minor, probably because other adhesion receptors (GPIb-IX-V and collagen-binding integrin $\alpha 2\beta 1$, for example) deliver necessary platelet adhesion and thrombus stability properties, and the vascular conditions permit an accentuated contribution to platelet activation from thrombin and other soluble agonists.[79,82,83] Keeping this in mind, the findings imply overall that GPVI may be a reasonable target for antithrombotic therapies as its nonessential role in hemostasis but important role in thrombosis means that the therapeutic targeting is unlikely to lead to unacceptable bleeding.

Existing clinical strategies to target platelet adhesion/activation

Numerous clinical therapeutics exist that target platelet activation and platelet adhesiveness (Table 2).[84,85] The majority of the therapeutics aims to block the receptors involved in the initial and amplification stages of platelet activation; several reagents are often used in combination to achieve a potent antiplatelet effect. Dual and triple antiplatelet therapies have been shown to prevent ischemic events in high-risk patients with coronary artery disease or during percutaneous coronary interventions, but they can cause bleeding complications.[8] Further, the incidence of recurrence of adverse vascular events remains of concern. The range of currently approved reagents includes:

- Anticoagulants, such as lepirudin, warfarin, unfractionated heparin, low molecular weight heparin, bivalirudin, edoxaban, rivaroxaban, argatroban, and dabigatran[86,87] that are direct or indirect inhibitors of thrombin and so block coagulation and interfere with the thrombin engagement of protease-activated receptors (PARs) on platelets
- Clopidogrel, prasugrel, and ticagrelor,[88] which serve to block platelet ADP receptor $P2Y_{12}$
- Aspirin, which blocks thromboxane generation (a strong platelet agonist) via inhibition of cyclooxygenases
- Abciximab, eptifibatide, and tirofiban,[89] which target fibrinogen interactions with the platelet-specific fibrinogen receptor $\alpha IIb\beta 3$.

The main issue with these reagents is the elevated risk of adverse bleeding, due to the therapeutics targeting molecular pathways that are important for both thrombotic processes and normal hemostasis.[90] This issue is compounded because the assessment of bleeding risk in patients receiving one or more of these therapies is complicated,[91,92] generally requiring specialized

Table 2 Examples of existing and novel therapeutics targeting platelet receptors

Platelet receptor	GPIbα	GPVI	PAR-1	$P2Y_{12}$	Thromboxane receptor	αIIbβ3
Copy number/platelet	18,000–25,000	6,000–10,000	500–2,000	Undefined	Undefined	60,000–80,000
Endogenous ligand	Shear-exposed VWF, P-selectin, αMβ2	Subendothelial collagen, collagen fragments	Thrombin	ADP	Thromboxane	Fibrinogen, VWF, collagen
Phase of hemostasis	Primary	Primary	Primary	Amplification	Amplification	Stabilization
Existing therapeutics that directly target this receptor				Clopidogrel, Prasugrel, ticagrelor		Abciximab, tirofiban, eptifibatide
Investigational therapeutics	6B4-Fab	Revacept, kistomin, glaucocalyxin	Vorapaxar, atopaxar	Cangrelor, cilostazol	Terutroban	

Abbreviations: GP, glycoprotein; PAR-1, proteinase-activated receptor 1; VWF, von Willebrand factor; ADP, adenosine diphosphate; Fab, fragment antigen-binding.

platelet function analysis where the relationship between platelet function testing and bleeding in different patient groups on combinational therapy is not clear, due to limited data.

Targeting GPVI therapeutically

GPVI has emerged as a potential target for antithrombotic therapy for a number of reasons. First, GPVI is only expressed on platelets and megakaryocyte (platelet precursor cells in bone marrow) populations[17] in relatively low abundance,[93] thus permitting high specificity while minimizing potential side effects of a therapeutic agent. Second, GPVI appears to play only a supporting role in normal hemostasis,[72,94] implying that targeting GPVI would not increase bleeding risk to unacceptable levels. Third, in animal models of thrombosis as well as in studies of inflammation[95] and reperfusion injury following ischemia,[96,97] a significant contribution of GPVI to tissue injury – together with experiments demonstrating the benefits of blockade of GPVI to reduce the extent of injury[16] – have been well-documented.

Several options exist to modulate or inhibit GPVI-mediated platelet activation. GPVI–collagen interaction can be disturbed by collagen-binding molecules (GPVI mimics), by GPVI-function blocking reagents (aptamers, small molecules, and antibodies), or by GPVI depletion.

GPVI mimetics

Taking advantage of the stronger collagen-binding affinity of dimeric GPVI, a recombinant fusion protein was formed between the extracellular collagen-binding domain of GPVI and the C-terminal of human immunoglobulin Fc domain to form a soluble dimeric GPVI (GPVI-Fc).[98] This reagent specifically bound to collagen with high affinity and attenuated platelet adhesion to immobilized collagen in vitro and to sites of vascular injury in vivo.[98]

Importantly, at doses sufficient to reduce platelet adhesion, the soluble form of GPVI only moderately prolonged tail bleeding times.[99,100] Such a reagent holds several advantages over other types of GPVI inhibitors. The GPVI-Fc predominantly targets the exposed subendothelium at a site of vascular injury, suggesting that collagen exposed within a damaged vascular site is the primary site of binding. By directly targeting the site of interaction, there is no requirement for prolonged systemic inhibition of platelet function.[99] Further, there have been no reports of aberrant platelet activation, loss of GPVI on circulating platelets, or thrombocytopenia associated with the use of this reagent in animal models. By the addition of an appropriate molecular tag to GPVI-Fc, it is possible that this reagent may also be developed as a bioimaging tool as it selectively binds to presumably prothrombotic regions of a vascular bed that is enriched for collagen.[16,98,101]

Injection of GPVI-Fc (Revacept; Janssen-Cilag GmbH, Neuss, Germany) has improved endothelial dysfunction and vascular morphology in atherosclerotic rabbits[100] and reduced the cerebral infarct size and edema after ischemic stroke, with improved functional and prognostic outcome without intracranial bleeding.[102] In a Phase I study, Revacept efficiently inhibited collagen-induced platelet aggregation ex vivo, with no alteration of primary hemostasis in 30 healthy donors;[103] however, GPVI-Fc had only limited antithrombotic effects in animal models where the direct blockade of GPVI function was effective in preventing occlusive thrombus formation.[104]

While the experimental and early phase trial results with this fusion protein are encouraging, the precise clinical setting and the appropriate dosing and treatment regimens where this reagent may be useful still remain to be defined. Also, similar to other immunoglobulin fusion proteins and antibody therapies, the possibility of immunogenicity with repeated injections of Revacept remains a potential hazard.

GPVI-function blocking reagents

Anti-GPVI antibodies are of great interest as candidate antithrombotic reagents as they may be able to accomplish the dual purpose of interfering with collagen-GPVI interactions as well as triggering GPVI shedding and/or internalization. Numerous antibodies raised against human GPVI now exist,[10,35] most of which activate platelets either directly through GPVI engagement or indirectly via the interaction with FcγRIIa when the antibody is intact. The fragment antigen-binding (Fab) fragments of most of these antibodies, including 9O12, 5C4, 1G5, and OM2 have strong-to-very-strong affinity for GPVI and inhibit GPVI-induced platelet activation. Single domain antibody clones,[105] consisting of single heavy and light chain variable domains (11–13 kDa) and single chain antibodies,[106] are highly stable and protease-resistant reagents that can be humanized and readily expressed in phage display libraries. As monomers, these reagents also have the advantage of not clustering or crosslinking GPVI, causing unwanted platelet activation.[105,107]

The signaling pathways utilized by GPVI to transmit activation signals (Figure 2) may also be targeted therapeutically. Existing reagents targeting Syk[108] or Btk[109] – which are already approved as antitumor reagents for use in patients with lymphoma – have shown strong efficacy in blocking platelet-collagen responses via GPVI.

Interestingly, the anticancer histone deacetylase inhibitors have additional effects on platelet GPVI. They reduce the surface and total expression of GPVI and impair GPVI signaling, which results in the inhibition of the final common pathways of platelet activation.[110] New approaches that target GPVI-mediated reactive oxygen species[58] or limit GPVI signaling via the activation of nuclear receptors (liver X receptors) on platelets that dampen platelet-collagen responses[111] may represent novel, more subtle approaches to diminish but not block GPVI-signaling function. The fact that many of these agents have been used therapeutically in patients and do not seem to cause clinically significant bleeding provides encouragement and possibly underscores the relatively minor role for GPVI in normal hemostasis.

Final remarks

For a number of reasons, GPVI presents as an attractive and feasible target to modulate platelet function. Peripherally, it is found exclusively on platelets, meaning that its modulation is less likely to lead to off-target effects. GPVI is accessible on the surface of platelets and tools and reagents exist to: 1) rapidly block the function or downregulate surface levels of the molecule; and 2) quantify changes in the surface levels of GPVI (by fluorescence-activated cell sorting [FACS]) and/or shed soluble GPVI in plasma (by enzyme-linked immunosorbent assay [ELISA] or bead-based immunoassay).

While the loss of GPVI does not appear to seriously disrupt normal hemostasis, pathological thrombus formation is significantly attenuated by targeting GPVI. Despite these positive milestones, it is valuable to remember that many proteins and gene products contribute to a given platelet phenotype in the complex human system. In the case of mouse GPVI, at least one or more unknown modifier genes in a *modifier of hemostasis* locus were shown to control the extent to which platelet thrombus formation in vivo was disrupted by the absence of platelet GPVI. Conceivably this could occur by altering the composition and thrombotic nature of the extracellular matrix through the regulation of gene expression in endothelial cells, smooth muscle cells, and/or fibroblasts.[112]

Further, GPVI-based inhibitors need to be carefully evaluated for safety, efficacy, and potency in the different patient groups and – as a monotherapy – suitability for acute conditions, or in combination with the existing antiplatelet and anticoagulant therapies as part of an approach to chronic treatment. For these reasons, data from human trials are eagerly awaited. However, together with new tools to specifically examine the antithrombotic (and other) effects of new and existing anti-GPVI reagents, such as a mouse expressing human GPVI,[113] a reagent that controls GPVI expression and function is practical and a reasonable proposition.

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Disclosure

The authors report no conflicts of interest in this work.

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