Implications of glycoprotein VI for theranostics

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Summary

Glycoprotein VI (GPVI), a membrane glycoprotein solely expressed in platelets and megakaryocytes, plays a critical role in thrombus formation due to collagen/GPVI-mediated platelet activation and adhesion. Recent studies have shown that surface expression of GPVI on circulating platelets is enhanced in acute cardiovascular diseases such as myocardial infarction and ischaemic stroke. Increased GPVI levels are associated with poor clinical outcome and are an early indicator for imminent myocardial infarction in patients with chest pain. The soluble form of the dimeric GPVI fusion protein (sGPVI-Fc) binds with high affinity to collagen and atherosclerotic plaque tissue. Non-invasive imaging studies with radiolabelled sGPVI-Fc show specific binding activity to vascular lesions *in vivo*. Further, sGPVI-Fc has been developed as a new therapeutic platelet-based strategy for lesion-directed antithrombotic therapy. This review summarises the potential of GPVI for diagnostic and therapeutic options based on novel non-invasive molecular imaging modalities to ameliorate care of patients with cardiovascular diseases.

Keywords

Platelets, glycoprotein VI, biomarker, myocardial infarction, stroke, molecular imaging

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Introduction

Platelets are essential for primary haemostasis and thrombosis at sites of vascular injury (1, 2). In atherosclerotic lesions, extracellular matrix proteins are exposed towards the blood stream and propagate thrombosis (3). Fibrillar collagen is the major extracellular matrix protein in artery walls. Circulating platelets adhere to collagen, become activated and initiate thrombus formation. Glycoprotein VI (GPVI) is surface-expressed on platelets and is the prominent receptor that mediates platelet adhesion to collagen (4). GPVI is a type I transmembrane protein belonging to the immunoglobulin superfamily and is uniquely expressed in platelets and megakaryocytes (4–6). Up to 9,600 copy numbers of GPVI expressed per platelet have been estimated in a quantitative analysis of platelet protein (7). Whereas the dimeric form of GPVI binds with high affinity to collagen, the monomeric form does virtually binds with low affinity (8). Platelet activation by adenosine diphosphate (ADP) or thrombin receptor-activating peptide (TRAP) results in enhanced surface expression of GPVI on the plasma membrane and induces dimerisation of the receptor (9). Further, activated coagulation factor X (FXa) and high-shear forces may induce cleavage and shedding of the GPVI ectodomain dependent on metalloproteinases of the a disintegrin and metalloproteinase (ADAM) family (10–12) leading to enhanced plasma concentrations of soluble GPVI. Thus, GPVI has raised great interest in cardiovascular science for its antithrombotic potential (13).

Both direct inhibition of platelet-associated GPVI through specific antibodies as well as competitive inhibition of GPVI binding to immobilised collagen through soluble dimeric GPVI have been shown to inhibit arterial thrombosis with limited risk of bleeding *in vivo* (13-15). Due to the fact that GPVI is uniquely found on platelets and released as a soluble form into the plasma it has become an interesting diagnostic target for biomarker development, as well (16). Since GPVI-binding sites are primarily exposed on vulnerable vascular lesions to promote thrombus formation, soluble dimeric GPVI may be a convincing tool for molecular imaging of vulnerable plaques (17). Thus, the therapeutic and diagnostic (theranostic) potential of GPVI will make the receptor a promising target for the development of personalised strategies to improve efficacy of therapy in patients at high risk for atherothrombotic events such as myocardial infarction and ischaemic stroke.

GPVI as a thrombotic biomarker

Platelet hyperaggregability and activation are one of the key mechanisms involved in the atherothrombotic complications associated with coronary and cerebrovascular diseases (18). Activation of circulating platelets is enhanced in acute coronary syndrome (ACS) and ischaemic stroke which is associated with poor outcome (19, 20). Low response to clopidogrel in patients with coronary artery disease treated with percutaneous coronary intervention (PCI) showed an increased risk for thrombo-ischaemic events and death (21). Moreover, cardiovascular risk factors such as diabetes mellitus are associated with an enhanced collagen-mediated platelet activation (22).

GPVI expressed on platelet-surface in patients with myocardial infarction and stroke

Surface expression of GPVI on circulating platelets has been shown to be altered in cardiovascular diseases (23, 24) (▶Table 1). The platelet Fc receptor that forms a functional complex with GPVI was significantly increased in patients with diabetes mellitus compared to those without diabetes. Fc receptor expression correlated with GPVI expression and was found to be independently associated with diabetes mellitus (25). In a consecutive cohort of 367 patients with symptomatic coronary artery disease, patients with ACS showed significantly enhanced GPVI expression on platelets compared with patients with stable coronary artery disease (23). In this study, the expression levels correlated with platelet degranulation markers such as CD62 and were independent of markers of myocardial necrosis such as troponin and creatine kinase. Moreover, patients with increased GPVI expression on hospital admission for acute chest pain had a 1.4-fold relative risk for ACS independent on myocardial necrosis marker troponin (▶Figure 1) (26). GPVI may thus be considered as an early marker for imminent acute coronary events in patients with chest pain (27). Further, platelet-associated GPVI was found to be enhanced in patients with ACS and ambiguous electrocardiogram (ECG) (28). High GPVI levels were also associated with increased residual platelet aggregation despite conventional dual antiplatelet therapy (26). In a large prospective study enrolling 2,213 consecutive patients who presented with chest pain, elevation of platelet GPVI was associated with a poor clinical outcome for composite events such as myocardial infarction, stroke, and cardiovascular death (29). Similar to ACS, patients with transient ischaemic attack (TIA) or ischaemic stroke showed enhanced expression of platelet GPVI (30). On patient hospital admission, enhanced GPVI levels were associated with a 2.4-fold relative risk for stroke and therefore with poorer clinical outcome in cumulative event-free survival for stroke, myocardial infarction, and cerebrovascular death at three-month follow-up (▶Figure 1). However, an increased affinity or avidity of the respective antibody used for detection of platelet GPVI following platelet activation might also contribute to the observed enhanced effects of GPVI expression. Conformational changes of GPVI such as receptor dimerisation have been shown to increase binding of platelets to collagen (9, 31). Thus, influence of such receptor modifications on binding of specific antibodies against platelet GPVI needs to be investigated in further studies.

Plasma levels of soluble GPVI (sGPVI) in cardiovascular diseases

Upon platelet activation, GPVI is strongly expressed on the platelet surface and partially cleaved and shed from the plasma mem-

Table 1: GPVI as a biomarker for cardiovascular diseases.

Marker	Usage	Target	Reference
pGPVI	diagnostic	AMI	(59)
pGPVI	diagnostic/prognostic	SAP/ACS	(23),(26)
sGPVI	diagnostic	Alzheimer's Disease	(37)
pGPVI	diagnostic	Atrial Fibrillation/ACS	(24)
pGPVI	diagnostic	Platelet Count/ACS	(60)
pGPVI	diagnostic	Chest pain/ACS	(27)
pGPVI	diagnostic	Ambiguous ECG/AMI	(28)
pGPVI	diagnostic/prognostic	Stroke/TIA	(30)
pGPVI	prognostic	SAP/ACS	(29)
sGPVI	diagnostic	Stroke	(36)
sGPVI	diagnostic	DIC	(10)
sGPVI, pGPVI	diagnostic	SAP/ACS	(33)
sGPVI	therapeutic	ACS	(15)
sGPVI	diagnostic	Stroke	(61)

AMI – acute myocardial infarction; SAP – stable angina pectoris; ACS – acute coronary syndrome; ECG – electrocardiogram; TIA – transient ischemic attack; DIC – disseminated intravascular coagulation; pGPVI – platelet glycoprotein VI; sGPVI – soluble glycoprotein VI.

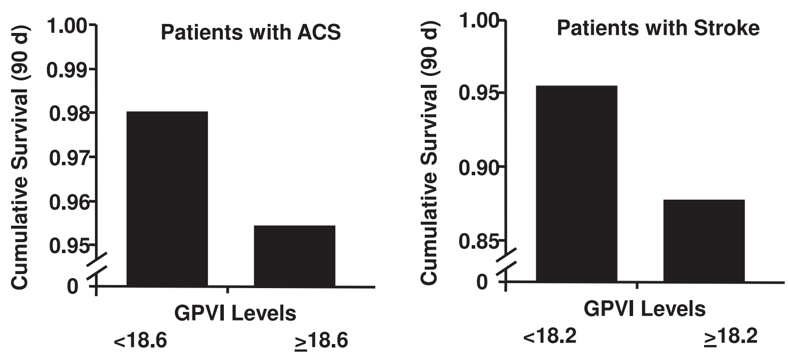

Figure 1: Platelet surface expression of glycoprotein VI and survival at a three-month follow-up for patients with ACS and stroke. A) Patients with symptomatic coronary artery disease and elevated platelet collagen receptor glycoprotein VI (GPVI) expression (mean fluorescence intensity (MFI) ≥ 18.6) had a poorer clinical outcome in composite cumulative survival that included myocardial infarction, stroke, and cardiovascular death than patients with a decreased GPVI expression (log rank; p = 0.002) (modified according to (29)). B) These results were paralleled in composite cumulative survival in patients with ischaemic stroke at an MFI ≥ 18.6 (log rank; p = 0.045) (modified according to (30)).

brane as a soluble form of GPVI (sGPVI). Metalloproteinases ADAM 10 and ADAM 17 (11, 12), coagulation factor Xa (10), and high shear forces (32) have been documented to contribute to this cleavage and shedding process. Thus, increased levels of sGPVI can have different pathophysiological causes, and utilizing sGPVI as a biomarker for platelet-associated cardiovascular diseases might give us new insights for the diagnostic process. Recently, several sensitive detection reagents and assays for sGPVI have been developed (33). Elevated plasma levels of sGPVI have been described in patients with immune thrombocytopenia purpura (ITP) (34), lupus nephritis (35), dissiminated intravasal coagulation (DIC) (10), stable coronary artery disease (33), and acute ischaemic stroke (36). Decreased levels of sGPVI have been described in patients with Alzheimer disease (37) and with atrial fibrillation (24). Platelet activation is known to play a prominent pathophysiological role for disease progression and, thus, a platelet- and plasma-based GPVI biomarker analysis may be a promising strategy to identify the state of cardiovascular diseases (acute vs stable). However, prospective and interventional studies are needed to further substantiate utility of GPVI for diagnostic purposes and risk managment.

GPVI as molecular tool for imaging

Introduction of biomarkers in the field of thromboischaemic diseases such as coronary and cerebrovascular diseases have significantly improved patient care in cardiovascular medicine. Although determination of biomarkers allows definition of the individualised risk, the consequences for therapy are limited. Molecular imaging has the great potential to combine the biological assessment of vascular lesions with imaging tools to localise areas at risk within the vascular branch. Conventional imaging modalities to define atherosclerotic vessel disease and luminal stenosis are of poor prognostic value for the prediction of myocardial infarction (MI) or stroke. The majority of MIs are caused by vulnerable plaques with a lumen narrowing <70% (38) that are rupture-prone or characterised by rapid progression and hardly detectable by conventional imaging tools (39). Besides being rich in lipids, inflammatory cells or metalloproteinases, vulnerable plaques are prone to platelet adhesion and thrombus formation and thus, trigger clinically relevant thromboischaemic events often in the absence of severe stenosis. Detection of vascular lesions *at risk* using molecular imaging may open the gate to adapted early preventive strategies.

In the past, recent approaches using novel molecular probes and radionuclide imaging have been developed to detect plaque instability of vascular lesions (40). Thrombogenecity of atherosclerotic plaques may be detected by using radiolabelled GPVI. The soluble dimeric form of GPVI can be fused to the human immunoglobulin Fc domain (GPVI-Fc), generated and purified as recombinant protein, and is characterised by high affinity to collagen (41). GPVI-Fc binds to collagenous structures in the core region of human atheromatous plaque (42) and to vascular lesions in mice (43). Radioiodinated GPVI and *in vivo* scintigraphy have been shown to be sensitive and non-invasive imaging modalities to detect thrombogenicity of vascular lesions in mice (17) (▶Figure 2A, upper panel). In a mouse and rabbit model of carotid artery injury, acute lesions could be detected after systemic administration of fluorescence-labelled GPVI and subsequent optical imaging (44, 45). Binding of GPVI-Fc-FITC to collagen, in both models, could be inhibited by unlabelled GPVI-Fc administered prior to injection of GPVI-FITC.

Preliminary experiments of PET imaging revealed an increased uptake of ^{124}I-GPVI-Fc in the aortic arch of high-fat diet ApoE$^{-/-}$ mice compared to wild-type (WT) mice (▶Figure 2A, lower panel). To allow clinical translation of this approach, compared to ^{124}I and other PET isotopes, ^{64}Cu may be preferred due to its improved spatial resolution (higher image quality) and adequate half life time (12.7 hours) for delayed PET studies (46). In a murine model of high-fat diet for 12 weeks, we found that the 64Cu-GPVI-Fc uptake in the aortic arch as evaluated by PET imaging was significantly increased in areas of atherosclerotic lesions in ApoE-/- compared to WT mice (47). Further, we showed in a PET/CT imaging study using ^{64}Cu-GPVI-Fc that vascular lesions at access site of heart catherisation may be visualised by non-invasive *in vivo* imaging in human (unpublished) (▶Figure 2B). Thus, GPVI-binding PET imaging is a promising tool for non-invasive identification of unstable vascular lesions and to guide medical treatment on an individualised basis (▶Figure 2B).

GPVI as antithrombotic therapy for thrombo-ischaemic diseases

The role of GPVI for haemostasis has been first described in a patient with mild bleeding tendency and deficiency of GPVI (48). Subsequent studies suggested that GPVI might be a promising antithrombotic target (13). In *in vivo* thrombosis models, GPVI could be established as an effective target to prevent platelet-dependent thrombus formation (41). Most research activities have concentrated on antibody-based strategies (14, 49–51) to inhibit the platelet-associated GPVI which are reviewed elsewhere (13).

Another approach to interfere with GPVI-dependent platelet adhesion and thrombus formation is to competitively block GPVI binding sites on immobilised collagen exposed on the vessel wall at sites of atherosclerotic lesions. Therefore, soluble dimeric fusion protein GPVI-Fc has been generated that binds with high affinity to collagen and atherosclerotic tissue (41, 42). Infusion of GPVI-Fc abolished stable arrest, aggregation of platelets and thrombus formation following vascular injury in mice (41) and rabbits (15, 52) without impact on bleeding times. Further, prolonged administration of GPVI-Fc attenuated atheroprogression (42, 53) and arterial remodelling after mechanical injury in ApoE$^{-/-}$ mice (43). In a murine disease model, administration of GPVI-Fc reduced infarct size and preserved myocardial function after transient ischaemia (54) and improved functional and prognostic outcome in ischaemic stroke without intracranial bleeding (55). In a phase I study, GPVI-Fc (Revacept®) has been shown to be a safe and well-tolerated compound that dose-dependently inhibited collagen-induced platelet aggregation without being accompanied by significant side effects or affecting general haemostasis or coagulation (56). A phase II study (clinicaltrials.gov/NCT01645306) evaluating the safety and feasability of Revacept® in patients with symptomatic carotid artery disease scheduled for surgical atherectomy just started and will provide further clinical data. Moreover, it may

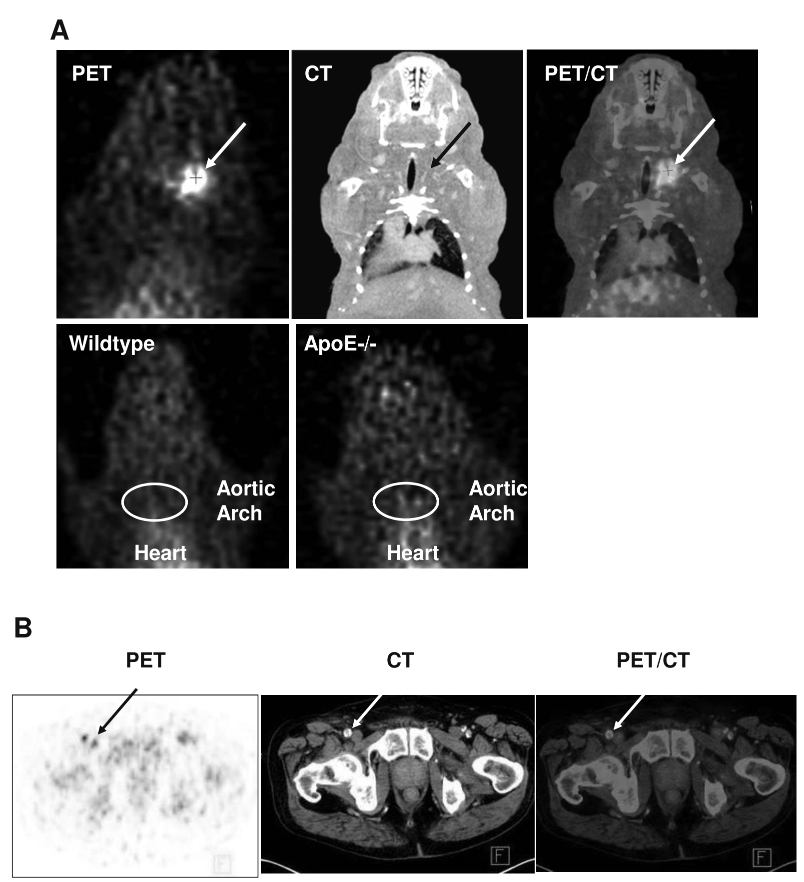

Figure 2: Targeted GPVI molecular imaging. A) Upper panel: ^{124}I-GPVI-Fc PET/CT imaging of injured *A. carotis comm.* in mice. C57BL/6J mice were administered ~ 7 MBq of ^{124}I-labelled GPVI-Fc directly after ligation induced injury of the left *A. carotis comm.* Imaging with animal microPET and microCT scanner 24 hours after tracer injection revealed an uptake of ^{124}I labelled GPVI-Fc at the lesion site. Lower panel: PET imaging of wild-type and cholesterol-fed 20-week-old ApoE$^{-/-}$ mice: 24 hours after intravenous injection of ^{124}I-GPVI-Fc an increased uptake in the aortic arch of ApoE$^{-/-}$ mice can be detected. B) 64Cu-GPVI-Fc PET/CT imaging of the postraumatic right *A. fem. comm.* after cardiac catherisation in humans. One week after cardiac catheterisation, a 79-year-old male patient with a significant coronary artery disease was administered ~140 MBq of ^{64}Cu-labelled GPVI-Fc. 24 hours after tracer injection, an uptake of ^{64}Cu labelled GPVI-Fc may be seen in the postraumatic right *A. fem. comm.* The signal-to-noise ratio (SNR) in the region of interest (ROI) of the posttraumatic right *A. fem. comm.* is 4.1 (calculated from the ratio of the standardised uptake value (SUV) mean to standard deviation in the ROI). The correspondent value of the SNR in the non-traumatic contralateral *A. fem. comm.* is 2.2 (unpublished personal communications).

be of future interest to monitor therapeutic effects of Revacept® (GPVI-Fc) performing competitive experiments with the novel molecular imaging tracer ^{64}Cu-GPVI-Fc.

Since GPVI-Fc binds to areas of vascular and tissue injury, further pharmacological concepts have been followed using GPVI-Fc fused to a second binding motif such as CD133 or CXCL12 (SDF-1). Both bispecific CD133-GPVI and SDF1-GPVI fusion proteins preserved myocardial function and enhanced neovascularisation following transient myocardial ischaemia (57, 58). These developments will help us elaborate new soluble dimeric GPVI proteins as injury-directed therapeutic options to facilitate repair and regeneration of diseased organs.

Conclusions

Recent work shed light into the importance of platelet GPVI for cardiovascular diseases. Platelet-associated and soluble plasma GPVI has been elaborated as novel biomarkers in various disease settings indicating a potential usefulness to assess thrombotic activity and response to antiplatelet therapy in patients. The soluble dimeric form of GPVI binds with high affinity to collagen and can visualise vulnerable vascular lesions *in vivo* using non-invasive PET imaging modalities. Further, soluble GPVI is a feasible and promising strategy to develop a lesion-dericted antithrombotic therapy in patients with an enhanced risk of bleeding. Ongoing clinical studies evaluating the significance of diagnostic therapy with the target GPVI are needed to develop promising strategies for individualised cardiovascular medicine (*theranostics*).

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Conflicts of interest

Meinrad Gawaz is a cofounder of the spin-off biotech company of the University of Tübingen and Würzburg, AdvanceCor, Martinsried, Germany. The company developed Revacept® (GPVI-Fc).

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