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REVIEW

Platelet-vessel wall interaction in health and disease

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ABSTRACT

Upon vessel wall injury platelets rapidly adhere to the exposed subendothelial matrix which is mediated by several cellular receptors present on platelets or endothelial cells and various adhesive proteins such as von Willebrand factor, collagen and fibrinogen. Subsequent platelet activation results in the recruitment of additional platelets and the generation of platelet aggregates forming a stable platelet plug. In addition, activated platelets form a strong link between primary and secondary haemostasis as they provide the phospholipid surface that is necessary for the assembly of activated coagulation factor complexes required for thrombin generation.

Other than the physiological function acting as a first line of defence against bleeding, platelets may also contribute to pathological thrombus formation. Platelets play an important role in thromboembolic diseases and may contribute to the formation of occlusive thrombi which can lead to severe complications such as stroke or myocardial infarction.

Improved understanding of the respective roles of the various cellular receptors, adhesive proteins and regulatory proteins involved in platelet-vessel wall interaction and subsequent thrombus formation, both under physiological and pathological conditions, has led to the development and investigation of a broad range of antiplatelet drugs. This review provides an overview of the current knowledge on the mechanisms involved in the interaction between platelets and vascular endothelium and discusses recent advancements in the development of drugs interfering with platelet-vessel wall interaction at various stages of thrombus formation.

KEYWORDS

Adhesion molecules, antithrombotic agents, platelets, (sub) endothelium, thrombus formation, von Willebrand factor

INTRODUCTION

Platelets are circulating blood cells that do not interact with the intact vessel wall under normal circumstances. However, when the vessel wall is injured and the endothelium is disrupted, a rapid and complex interaction between circulating platelets and exposed (sub) endothelial structures occurs.[1] This interaction, mediated by various cellular receptors on the surface of platelets or endothelial cells and adhesive proteins such as von Willebrand factor (vWF) and fibrinogen, ultimately results in the adhesion of platelets to the vessel wall followed by aggregation of platelets to each other. In addition to this physiological function as a first line of defence against bleeding, platelets may also contribute to pathological thrombus formation. In systemic inflammatory syndromes such as sepsis, disseminated intravascular platelet activation may lead to microvascular thrombosis as well as an amplification of the inflammatory response through the release of inflammatory cytokines and growth factors. Furthermore, activated platelets form a strong link between the processes of primary and secondary haemostasis as they provide the phospholipid layer necessary for the assembly of activated coagulation factor complexes which in turn is required for thrombin generation and subsequent conversion of fibrinogen into fibrin.

This review provides a comprehensive overview of what is currently known about the mechanisms involved in platelet-vessel wall interaction, both under physiological and pathological conditions. The respective roles of various important cellular receptors, adhesive proteins and regulatory proteins, as well as the development of drugs interfering with platelet-vessel wall interaction at different levels of thrombus formation will be discussed.

ROLE OF PLATELETS IN NORMAL HAEMOSTASIS

Upon vessel wall injury, rapid and complex interactions between circulating platelets and exposed (sub)endothelial structures occur[1] resulting in platelet adhesion to the damaged endothelium. The mechanism by which platelets adhere to the vascular wall to achieve haemostasis is fairly well understood, with vWF-mediated platelet adhesion being the most important route, particularly in situations of high shear stress such as small arteries, arterioles and stenosed vessels. VWF is an adhesive glycoprotein synthesised by megakaryocytes and endothelial cells and is either constitutively secreted or targeted to storage organelles in the endothelium, called Weibel-Palade bodies, or to α-granules which are present in platelets or megakaryocytes.[2] The mature vWF molecule consists of disulphide-linked multimers with a molecular weight of 20,000,000 or more.[3] Under normal conditions vWF circulates in plasma in its inactive form whereas the largest and most active forms, high molecular weight (HMW) or ultra-large vWF multimers, are present in storage organelles and not found in circulating blood. However, upon endothelial cell activation vWF is acutely released from endothelial storage sites and can be detected transiently as ultra-large vWF in the circulation.[4,5] Subsequent binding of vWF to the exposed sub-endothelial structures, such as collagen, induces a conformational shift in the vWF molecule from its latent to its active form, thereby exposing the binding site for platelet glycoprotein (GP) receptor Ib. Through the simultaneous binding of collagen and platelets vWF can serve as a molecular bridge between platelets and the sub-endothelial matrix mediating platelet adhesion to the vessel wall.[6]

In addition to its important role in platelet adhesion, vWF may also be a ligand for the major platelet integrin αIIbβ3 (GPIIb/IIIa) thereby facilitating platelet aggregation. When platelets bind to vWF they become activated and undergo a conformational change. This shape change induces several effects, including the acute release of the contents of storage organelles, such as fibrinogen and ADP, further enhancing platelet activation.[7] Second, the conformational change induces the expression of active GPIIb/IIIa on the surface of activated platelets through which platelets can bind to either vWF or fibrinogen forming platelet aggregates.[8] Finally, the platelet membrane turns into a phospholipid surface upon activation and shape change. Since this negatively charged surface is necessary for the assembly of activated coagulation factor complexes which in turn is required for thrombin generation, platelets form a strong link between the processes of primary and secondary haemostasis. Secondary haemostasis ultimately results in the formation of a fibrin network stabilising the growing platelet thrombus.

Another adhesive protein crucially involved in platelet-vessel wall interaction is collagen. Collagen types I and IV may directly bind to platelet glycoprotein receptor Ia/IIa (integrin α2β1).[9] The importance of this GPIa/IIa mediated collagen-platelet binding seems limited to low shear rate conditions. In addition GPIa/IIa binding to collagen may also facilitate the interaction between collagen and another platelet glycoprotein receptor, GPVI. GPVI belongs to the immunoglobin superfamily, which forms an important group of cellular adhesive receptors. Although GPVI may be directly involved in platelet adhesion to collagen, it seems likely to predominantly act as an activator of the GPIa/IIa receptor via intracellular signalling.[10]

Other adhesive proteins present in the extracellular matrix that are involved in the interaction between platelets and the (sub) endothelium include fibronectin, thrombospondin, laminin and vitronectin. Fibronectin is produced by megakaryocytes. It is stored in platelet α-granules and secreted upon thrombin-mediated platelet activation. Through binding to platelet receptor GPIIb/IIIa fibronectin can mediate platelet-platelet interaction.

Another protein that is stored in α-granules and secreted upon platelet activation is thrombospondin, which binds to the platelet membrane where it can interact with fibrinogen, fibrin, fibronectin, collagen and other platelets. The physiological function of its release during platelet activation might lie in its capability to overcome the antithrombotic activity of physiological nitric oxide, thereby providing a positive feedback for efficient platelet adhesion and aggregation.[11] Binding of thrombospondin to platelets is mediated by platelet GPIV receptor. Other receptors that are possibly involved in the thrombospondin-platelet interaction include integrin α5β3 and GPIb.[12]

A third adhesive protein located in the extracellular matrix that is involved in the interaction between platelets and endothelial structures is the large glycoprotein laminin. Similar to collagen laminin can amplify platelet activation through GPVI binding. Recent data suggest that laminin may also interact with vWF and the GPIb/IX/V complex thereby supporting platelet adhesion under high shear stress conditions.[13] The extracellular adhesive protein vitronectin can bind to platelet receptor GPIIb/IIIa or the integrin αvβ3 and appears to be functionally similar to fibronectin.[14]

The superfamily of selectins, including L-selectin (expressed on leucocytes), E-selectin (expressed on endothelial cells) and P-selectin (expressed on both platelets and endothelial cells), forms an important group of cellular adhesive receptors. P-selectins are of particular importance for the interaction between platelets, leucocytes and the blood vessel wall. Platelet and endothelial cell activation induces the release of P-selectins from platelet α-granules and endothelial Weibel-Palade bodies[15] which

then become integrated into the cell membrane and mediate both platelet and leucocyte adhesion. Via the induction of tissue factor expression on circulating monocytes, P-selectin may promote fibrin formation as tissue factor is the primary initiator of thrombin generation which ultimately results in the conversion of fibrinogen to fibrin.[16] Increased levels of soluble P-selectin have been demonstrated during acute coronary syndromes as well as systemic inflammation.[16]

ROLE OF PLATELETS IN THROMBOTIC DISEASE

In addition to the physiological role as a first line of defence against bleeding in response to vascular injury, platelets may also interact with the intact endothelium and contribute to pathological thrombus formation. Although key events leading to thrombus formation in normal haemostasis may be similar in pathological thrombus formation in diseases like stroke or myocardial infarction, factors such as altered shear rate and local dysfunction of endothelial cells, possibly in association with inflammatory mechanisms, appear to play important contributory roles.[17] In case of arterial occlusion, increased shear rate can induce circulating vWF to unfold, bind to platelets through GPIb/IX/V interaction and initiate GPIIb/IIIa-mediated platelet aggregation, which ultimately may result in complete occlusion of the respective artery causing heart attack or stroke.[18-20] Another example of platelet-vessel wall interaction contributing to disease is the development of atherosclerosis in which platelets appear to be crucial.[21,22] In apoE deficient mice platelets were demonstrated to adhere to the vascular wall of the carotid artery even before the invasion of leucocytes and the development of manifest atherosclerotic lesions.[21] Furthermore, platelet receptors GPIbα and IIb/IIIa were found to be the main mediators of platelet adhesion[21] and prolonged antibody-mediated blockade of the platelet GPIbα receptor was demonstrated to inhibit the accumulation of leucocytes in the vascular endothelium as well as atherosclerotic lesion formation. The latter was further supported in different mouse models showing that interruption of platelet-vessel wall interaction, through either antibody-mediated inhibition or by knocking out various platelet receptors including GPIIb, GPIbα and P-selectin, substantially reduces atherosclerotic lesion formation.[22-24] Subsequently, atherosclerotic plaques may rupture thereby exposing the underlying subendothelial structures which could lead to pathological thrombus formation through vWF-GPIb/IX/V and/or collagen-GPVI interactions.

Inflammation is yet another pathological condition in which platelets are important.[25] Platelet activation is common in inflammatory states, including cardiovascular diseases such as unstable angina and acute myocardial infarction,[26,27] and systemic inflammatory syndromes like sepsis where intravascular platelet activation may lead to microvascular thrombosis and contribute to organ failure, morbidity and mortality.[28] The development of severe cardiovascular complications and the outcome of cardiovascular interventions (e.g. percutaneous interventions or bypass surgery) can be actively initiated and influenced by platelets and can therefore also be reduced by effective platelet inhibition.[29,30] Activated platelets can interact with various cell types at the vascular wall, including endothelial cells, neutrophils, monocytes and endothelial progenitor cells. Under normal circumstances platelets will not interact with the intact endothelium; however, inflamed endothelial cells develop properties that make them adhesive for platelets. Various *in vitro* studies performed with human umbilical vein endothelial cells have shown that platelets adhere to activated human endothelial cells and that this interaction is mediated by platelet receptor GPIIb/IIIa, involving platelet-bound fibrinogen, fibronectin and vWF, as well as endothelial receptors, such as intercellular adhesion molecule-1 (ICAM-1), αvβ3 integrin and GPIb.[31-33] Furthermore, platelet activation induces a local release of platelet granule contents containing various potent inflammatory substances which further enhance the inflammatory response and alter chemotactic, adhesive and proteolytic properties of endothelial cells.[34,35] Under inflammatory conditions platelets within the blood stream can interact with circulating leucocytes and once recruited to the vascular endothelium attract these cells to the vascular wall. Leucocyte infiltration into the vessel wall requires multiple steps, involving adhesive and signalling events. These steps include selectin-mediated adhesion and rolling of leucocytes over the endothelium, integrin-mediated firm adhesion of leucocytes, leucocyte activation, and finally the actual infiltration of inflammatory cells into the endothelium.[36] The interaction between platelets, and both leucocytes and the vascular wall can occur in various sequences. First, platelets can form aggregates with leucocytes thereby promoting leucocyte recruitment, either by activating leucocyte adhesion receptors or by directly serving as a bridging molecule between leucocytes and the endothelium. When adhered to the vessel wall, platelets can attract leucocytes by releasing chemoattractants and providing an adhesive surface for leucocyte adhesion. During these complex interactions platelets, leucocytes and endothelial cells all become activated in a cascade-like fashion. An important role is reserved for P-selectin (briefly discussed above) and its main ligand, P-selectin glycoprotein ligand-1 (PSGL-1). These adhesion molecules were originally characterised to be important for initial rolling interactions between leucocytes and the vessel wall, which are required for subsequent leucocyte recruitment

to sites of inflammation or infection.[37,38] However, various animal studies using different models of thrombosis have revealed an important role for P-selectin and PSGL-1 in the process of thrombosis as well. Mice deficient in either of these molecules were demonstrated to form thrombi which were markedly reduced in mass, fibrin level and tissue factor accumulation. Furthermore, compared with wild type mice, P-selectin or PSGL-1 deficient mice were shown to have reduced numbers of infiltrating inflammatory cells in the affected vessel walls.[39,40] The observed differences in tissue factor expression and fibrin generation were already observed in the first 20 seconds after injury, prior to leucocyte recruitment. Similar results were obtained in wild-type mice treated with anti-P-selectin blocking antibodies prior to thrombosis induction and further support a crucial role for P-selectin and PSGL-1 in the recruitment of tissue factor bearing microparticles in early thrombi and subsequent fibrin formation.[41]

PROTEINS REGULATING PLATELET-VESSEL WALL INTERACTION

Although much information is available on mechanisms involved in platelet activation, little is known about the signalling pathways that negatively regulate platelet function. Here we will discuss two proteins importantly involved in the regulation of platelet-vessel wall interaction, platelet-endothelial-cell adhesion molecule-1 (PECAM-1) and von Willebrand factor cleaving protease ADAMTS13.

The immunoglobulin (Ig) gene superfamily consists for a large part of molecules that are involved in the recognition of adhering cells, including intercellular adhesion molecule (ICAM) 1 to 3, vascular adhesion molecule (VCAM) and platelet-endothelial cell adhesion molecule-1 (PECAM-1). In contrast to the cellular adhesion receptors ICAM and VCAM, which play important roles in the interaction between leucocytes and endothelial cells, PECAM-1 is not so much involved in mediating platelet-endothelial cell interaction, but rather acts as a negative regulator of platelet activation through the down modulation of platelet receptor GPIb/IX/V and GPVI. As discussed before, GPIb/IX/V and GPVI bind to vWF and collagen, respectively, and are involved in the initial interaction between circulating platelets and the vessel wall, either under physiological or pathological circumstances. Binding of vWF to the GPIb/IX/V complex appears to simultaneously activate PECAM-1, thereby forming a negative feedback loop. Various regulatory functions of PECAM-1 have been identified in murine platelets.[42] For one, PECAM-1 was shown to negatively regulate early GPIb-initiated platelet signalling responses. Second, PECAM-1 appeared to control the rate and extent of GPIb-mediated activation of platelet receptor GPIIb/IIIa. Furthermore, PECAM-1 was demonstrated to limit the size and rate of platelet thrombus formation under conditions of physiological flow. Via the down modulation of both GPIb/IX/V complexes and GPVI receptors, PECAM-1 can prevent unnecessary platelet activation under high shear and acts as a negative regulator of both platelet activation and aggregation.

VWF-mediated platelet adhesion forms the most important route for platelet-vessel wall interaction under conditions of high shear stress. VWF multimer size, and thus also vWF activity, is primarily regulated by the metalloprotease ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13), whereas recently other factors have also been identified to cleave vWF, such as plasmin and leucocyte proteases.[43-45]

ADAMTS13, sometimes also referred to as vWF cleaving protease, rapidly cleaves acutely released large vWF multimers into smaller fragments, thereby reducing its propensity to facilitate platelet adhesion and aggregation.[46] ADAMTS13 is synthesised in hepatic stellate cells, endothelial cells, megakaryocytes or platelets, and is enzymatically active in circulating blood.[47-50] However, under static conditions the specific cleavage site located in the vWF A2 domain (Tyr1605-Met1606) is buried within the vWF molecule which normally circulates in plasma in a globular form and thus cannot be recognised by its cleaving protease. Various circumstances can induce vWF unfolding, thereby exposing the ADAMTS13 cleavage site and increasing the proteolytic degradation of vWF by ADAMTS13. These include high shear conditions,[51] denaturing agents such as urea[52] and mutations as seen in the bleeding disorder von Willebrand disease type 2A.[53] In contrast, reduced ADAMTS13 activity may lead to insufficient vWF processing causing a prothrombotic state as is often observed in thrombotic thrombocytopenic purpura (TTP). TTP is a rare and life-threatening condition classified in the group of thrombotic microangiopathies in which a systemic microvascular aggregation of platelets can cause ischaemia in the brain, kidney and other organs, thrombocytopenia, and mechanical injury to erythrocytes.[54] TTP is strongly associated with a severe deficiency of ADAMTS13. The majority of cases are caused by a congenital or acquired (autoantibody-mediated) deficiency resulting in the presence of endothelial cell-attached ultra-large vWF multimers which readily bind to platelet receptor GPIb and promote platelet adhesion and aggregation.[55,56] Other factors that have been proposed to possibly inhibit ADAMTS13 activity include free haemoglobin, inflammatory cytokine IL-6, leucocyte elastase, thrombin, activated coagulation factor X and plasmin.[44,57,58] However, most data pointing to an inhibitory function of these proteins on ADAMTS13 activity are obtained *in vitro* and for free haemoglobin and IL-6 supraphysiological concentrations were used.

Recently, ADAMTS13 deficiencies have also been reported in non-TTP diseases that are accompanied by extreme endothelial activation, including severe sepsis, various inflammatory states and severe malaria.[59-61] However, the clinical relevance of these observations remains unsure.

DEVELOPMENT OF DRUGS INTERFERING WITH PLATELET-VESSEL WALL INTERACTION

Improved understanding of the roles of various platelet receptors and adhesive proteins involved in vascular cell adhesion has contributed to the development and evaluation of many antithrombotic agents interfering with either of these processes. In this section the various drugs targeting different pathways involved in platelet-vessel wall interaction will be discussed.

Drugs targeting vWF- GPIb/IX/V interaction

As was previously discussed, the initial adhesion of platelets to the damaged vessel wall depends, in particular under high shear stress conditions, on the binding of the platelet GPIb/IX/V complex to subendothelial bound vWF. Through platelet GPIb/IX/V-vWF interaction platelets start rolling over the stretched vWF molecules that line the damaged endothelium, which is followed by firm platelet adhesion and further thrombus formation at the site of vascular damage. One of the strategies pursued to inhibit thrombus formation is to hamper this initial step of platelet adhesion to thrombogenic surfaces by interfering with this vWF-GPIb/IX/V axis.

Clinical evidence for the role of vWF-GPIb/IX/V interaction in thrombus formation was found in patients affected with von Willebrand disease or Bernard Soulier syndrome, caused by abnormalities in the vWF molecule and the GPIb/IX/V complex respectively.[62] VWF plays a highly important role in normal haemostasis. As discussed, it promotes platelet adhesion and aggregation. In addition it serves as a stabilising carrier molecule for coagulation factor VIII.[2] Von Willebrand disease is the most common inherited bleeding disorder. Either qualitative defects (type 2) or quantitative defects (type 1 and type 3) in the vWF molecule lead to impaired vWF-GPIb/IX/V interaction, causing mucocutaneous bleeding and reduced circulating FVIII levels resulting in (spontaneous) bleeding tendencies as seen in haemophilia.[63] The autosomal recessive bleeding disorder Bernard Soulier syndrome is characterised by giant platelets, low platelet count and prolonged bleeding time. Affected patients clinically present with bleeding problems such as epistaxis, mucocutaneous, gingival or trauma-induced bleeding. Various mutations affecting different parts of the GPIb/IX/V complex have been indentified, but most mutations are found in the GPIb region resulting in decreased expression of platelet receptor GPIbα on the platelet surface.[64]

The clinical relevance of von Willebrand disease and Bernard Soulier syndrome emphasises the importance of vWF-GPIb/IX/V interaction in normal thrombus formation. Inhibition at this level appears to be an attractive approach for antithrombotic treatment as targeting the initial interaction between platelets and the vascular wall can be expected to give strong effects. Furthermore, the particular importance of vWF-GPIb/IX/V interaction for platelet adhesion under high shear stress conditions implies that drug-mediated interference at this axis will be more specific for arterial systems where shear stress is generally high. In contrast venous systems with lower shear rates can be expected to be less affected, resulting in fewer bleeding complications. Finally, the important roles for platelets (adhesion and activation) and thrombus formation in restenosis after angioplasty, which remains a significant clinical problem despite technical improvements, make the vWF-GPIb/IX/V axis an appealing target for antithrombotic therapy.[65] In particular since interference at this level will, in addition to platelet adhesion inhibition, also reduce subsequent platelet activation. This will result in decreased platelet granule content release, previously shown to affect thrombin generation as well as smooth muscle cell migration and proliferation, thereby leading to decreased fibrin deposition and neointima formation.[66]

Molecules that have been tested for inhibition of the vWF-GPIb/IX/V interaction *in vivo* include snake venoms, such as crotalin and agkistin, peptides derived from vWF (e.g. AR545C, RG12986 and VCL), and various monoclonal antibodies. The clinical applicability of the first two groups seems limited, as snake venoms have been shown to induce significant bleeding and vWF-derived peptides appear to have a short duration of action after administration.[67-72] In contrast, monoclonal antibodies (moAbs) interfering with vWF-GPIb/IX/V interactions appear to have an effective and long-term antithrombotic effect without affecting bleeding and thus seem most promising. MoAbs directed against either the vWF A1 domain or the GPIb/IX/V complex have been tested *in vivo* and have been reviewed elsewhere.[73]

Various moAbs directed against the vWF A1 domain involved in GPIb/IX/V binding have been identified, however, only a few have been reported to have *in vivo* antithrombotic efficacy. This is mainly due to the low cross-reactivity of the antibodies between different species and the common occurrence of pronounced bleedings. Nevertheless one monoclonal antibody, named AJvW2, which was first isolated from a murine moAb blocking the vWF A1 domain in 1997, generated successful results in several *in vivo* animal studies.[74-76] In line with these animal

data the first human clinical trial, testing the humanised antibody in healthy volunteers, demonstrated effective vWF-GPIb/IX/V inhibition without an effect on bleeding time or significant clinical adverse events.[77] Although these initial results are encouraging, additional clinical studies are needed to further investigate whether the antibody can serve as an effective and safe agent for antithrombotic treatment in humans.

Also several moAbs directed against GPIb have been developed and characterised *in vitro,* but again only little *in vivo* data exists. Again this is partly due to low cross-reactivity of the anti-GPIb monoclonal antibodies with GPIb of different species. Furthermore, severe thrombocytopenia and increased bleeding times were often observed (e.g. with AP-1, PG-1, PP4-3C, 6B4 IgG) hampering further use of these anti-GPIb moAbs *in vivo*.[78-80] However, the moAb 6B4 Fab was shown to have a strong antithrombotic effect in various *in vitro, in vivo* and *ex vivo* models in baboons without affecting platelet count or bleeding time.[80,81] Further studies with humanised 6B4 Fab are needed to confirm these results in humans and to determine the potential use of this compound for antithrombotic treatment in humans.

Drugs targeting collagen-vWF interaction

Collagen is one of the most thrombogenic subendothelial compounds triggering thrombus formation when it becomes exposed upon endothelial damage. Under low shear stress conditions platelets can directly bind to collagen, whereas under high shear rates binding of vWF to collagen is a prerequisite for platelet adhesion. The physiological importance of collagen-vWF interaction is emphasised by the clinical bleeding tendency observed in a family with impaired binding of vWF to collagen.[82] Due to the particular importance of vWF-collagen binding for platelet adhesion under high shear conditions, one can hypothesise that inhibition of vWF-collagen binding specifically decreases thrombus formation in situations of high shear stress, such as stenosed vessels, whereas haemostasis in healthy vessels will be less affected. Various compounds interfering with the binding of vWF to collagen have been developed and characterised, but only three have been evaluated for their effects *in vivo*. Although *in vitro* promising results were obtained for leech antiplatelet protein (LAPP), no effect was observed on thrombus formation in an *in vivo* baboon model.[83] In contrast, another leech protein named saratin was shown to reduce thrombus formation and stenosis without affecting platelet count or bleeding time in a rat model, and was demonstrated to reduce platelet deposition on human atherosclerotic plaques in a pig model.[84,85] *In vivo* testing of a monoclonal antibody inhibiting vWF-collagen binding (82D6A3) in baboons demonstrated a strong antithrombotic effect without significantly affecting bleeding time, platelet count or coagulation parameters.[86] Although these results seem encouraging, further studies with a humanised form of the antibody should be performed to confirm these data in humans.

Drugs targeting collagen-GPVI and collagen-GPIa/IIa interaction

The major collagen receptor GPVI is solely expressed on megakaryocytes and platelets. It plays important roles in both platelet adhesion and subsequent activation of integrins. Since GPVI deficiency, causing impaired platelet-collagen binding, leads to only mild bleeding problems,[87,88] the GPIV-collagen interaction might serve as an attractive and safe target for antiplatelet therapy. Several drugs have been developed of which some have also been evaluated *in vivo*. Both antimurine and antihuman GPVI monoclonal antibodies have been shown to inhibit thrombosis without significantly prolonging bleeding times in mice and monkeys, respectively.[89,90] The dimeric form of soluble GPVI was expected to serve as a competitive inhibitor *in vivo,* but appeared to have no antithrombotic effect when tested in a comparative study using soluble GPVI and anti-GPVI antibodies.[91] Surprisingly the peptide EXP3179, which is an active metabolite of the angiotensin II type 1 receptor antagonist losartan, was demonstrated to specifically inhibit GPVI-mediated platelet adhesion after acute vessel injury in mice.[92] Drug-mediated interference with collagen-GPVI interaction has clearly been shown to have antithrombotic potential; however, further research including clinical studies are needed to validate this approach.

Another important collagen receptor that is present on platelets and capable of binding different types of collagen is integrin $\alpha 2\beta 1$, or glycoprotein Ia/IIa. When platelets become activated, membrane bound GPIa/IIa receptors shift from a low affinity state to a conformation with high affinity for collagen binding. The clinical relevance of GPIa/IIa mediated platelet-collagen interaction emerged from the mild bleeding tendencies observed in GPIa/IIa deficient patients. Furthermore, *in vitro* experiments studying the effect of various GPIa/IIa antagonists on platelet adhesion and aggregation, demonstrated a contributory role for GPIa/IIa in thrombus formation.[93] However, opposite results were obtained in mice, with the observation of normal platelet adhesion and absence of bleeding problems in $\alpha 2$ or $\beta 1$ deficient mice.[94] Further studies, including in a non-human primate model, should be performed to determine whether the observed differences in bleeding in mice and man are due to species differences, and next, to confirm whether drugs targeting the GPIa/IIa-collagen interaction could have antithrombotic potential in humans.

Drugs targeting GPIIb/IIIa-fibrinogen or -vWF interactions

In contrast to the previously discussed compounds that inhibit collagen-vWF-GPIb/IX/V interactions

and thus hamper the initial step of platelet adhesion to thrombogenic surfaces, GPIIb/IIIa blocking agents interfere with the final step of platelet aggregation which is comprised of fibrinogen binding to platelet receptor GPIIb/IIIa. GPIIb/IIIa is the receptor that is most abundantly present on the platelet surface.[95] Upon platelet activation GPIIb/IIIa shifts from its latent to its active confirmation which enables the activated platelet receptor to interact with various adhesive proteins present in the plasma, including fibrinogen, vWF, vitronectin and fibronectin. Binding of platelets to other platelets however, is exclusively mediated by fibrinogen and vWF. Clinical evidence for the essential role of GPIIb/IIIa in platelet aggregation and thrombus formation in humans is provided by patients affected with the bleeding disorder, Glanzmann's thrombasthenia. In Glanzmann's thrombasthenia specific gene mutations cause an absence or non-functionality of the GPIIb/IIIa receptor resulting in impaired platelet aggregation which clinically presents with a life-long severe bleeding tendency. The potential antithrombotic effect of GPIIb/IIIa inhibition was demonstrated in both GPIIb/IIIa deficient mice (demonstrating a bleeding tendency similar to that seen in patients with Glanzmann's thrombasthenia) and mice treated with monoclonal anti-GPIIb/IIIa antibodies, which were shown to be protected from thrombosis in several thrombosis models using various thrombosis inducing stimuli.[96,97] A more detailed overview of the development of moAbs antagonising GPIIb/IIIa starting from the early 1980s is provided by De Meyer *et al.*[73] Although the first reports on the use of anti-GPIIb/IIIa moAbs were somewhat disappointing since side effects such as thrombocytopenia and significant bleeding were common,[98-101] more recent work has pointed to the beneficial effects of competitive GPIIb/IIIa-binding compounds in the prevention of platelet aggregation and thrombus formation. Although the GPIIb/IIIa antagonists abciximab, eptifibatide and tirofiban are currently approved by the FDA and used to manage myocardial infarction and coronary syndromes in humans, limitations remain, of which the risk of bleeding complications is the most important. One monoclonal antibody that might serve as an effective but safer alternative is the humanised antibody YM337 which recognises platelet glycoprotein IIb/IIIa specifically, whereas abciximab also binds other integrins such as $\alpha v\beta 3$ and $\alpha M\beta 2$. In both, animal and human studies, YM337 has been shown to effectively inhibit thrombus formation without affecting bleeding times.[102-104] However, in contrast to *ex vivo* antiplatelet experiments performed in rhesus monkeys, where a significantly smaller effect on template bleeding times was observed for YM337 compared to abciximab treatment,[105] no difference in bleeding time was observed in healthy human individuals treated with either YM377 or abciximab.[104] Further studies should be performed to confirm the potential use of YM337 as an antithrombotic drug in humans.

CONCLUSION

Platelet-vessel wall interactions play important roles in thrombus formation, either under physiological circumstances serving as a first line of defence against bleeding, or under pathological circumstances such as atherosclerosis or inflammation, where complex interactions between circulating platelets and the vascular wall can result in platelet adhesion, aggregation and finally pathological thrombus formation contributing to disease. Interactions between platelets and the vascular endothelium are mediated by various cellular receptors present on the surface of platelets and endothelial cells, adhesive proteins such as von Willebrand factor and fibrinogen, and important regulatory proteins including PECAM-1 and ADAMTS-13. The improved understanding of the different proteins involved in platelet-vessel wall interaction has led to the development of effective antithrombotic drugs targeting these processes at various levels. In the recent past several drugs targeting the collagen-vWF-GPIb/IX/V axis (interfering with collagen-GPVI or collagen-GPIa/IIa binding, or antagonising interactions between receptor platelet GPIIb/IIIa IPV and fibrinogen or vWF) have been developed and tested *in vivo*. Although encouraging results have been obtained and some drugs, including the GPIIb/IIIa-antagonists abciximab, eptifibatide and tirofiban, are currently approved by the FDA and used for the management of myocardial infarction and coronary syndromes in humans, some major limitations remain to be overcome of which the risk of (major) bleeding is the most important. Further studies should confirm whether the various developed compounds can actually serve as effective and safe alternatives for the treatment and prevention of thrombosis in humans.

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ERRATUM

Unfortunately in the photo quiz 'An 86-year-old man with a unilateral pectoral swelling' by Hunag-Bin Tasai[1,3], Chin-Chi Kuo[2,4], Jeng-Wen Huang[5*], Kuan-Yu Hung[5], which was published in Neth J Med. 2010;68(4):183, an error was made in the authors' names. The correct authors' names should have been:

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We apologise for any inconvenience.