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Molecular Interactions Involved in Von Willebrand Factor Mediated Platelet Adhesion Through Glycoprotein Ib-IX-V

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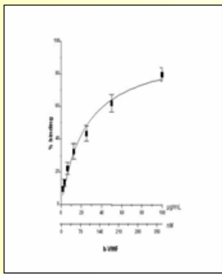
Aims of the Study

Upon damage of a blood vessel, platelets quickly form an initial haemostatic plug, resulting in the arrest of bleeding. Pathological conditions can lead to a deficient haemostasis which can result in bleeding disorders, or in the opposite thrombosis. Thrombosis remains the most common cause of death in developed countries and therefore a better understanding of haemostasis is essential for preventing and treating this disorder. The goals of this study were to further refine our understanding of the molecular mechanisms involved in the initial formation of a haemostatic plug, with special focus on the role of von Willebrand factor (VWF) and the platelet glycoprotein (GP) Ib-IX-V complex in this process.

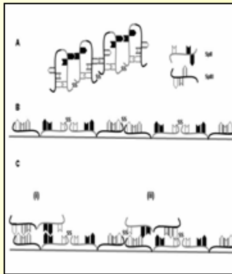
Results

The von Willebrand factor self-association is modulated by a multiple domain interaction *J.Thromb. Haemost.* 3, 552-61, 2005 IF 4.831

Platelet adhesion and aggregation at sites of vascular injury exposed to rapid blood flow require von Willebrand factor (VWF). VWF becomes immobilized by binding to subendothelial components or by a self-association at the interface of soluble and surface-bound VWF. As this self-association has only been demonstrated under shear conditions, our first goal was to determine whether the same interaction could be observed under static conditions. Furthermore, we wanted to identify VWF domain(s) important for this self-association. Biotinylated VWF (b-VWF) interacted dose-dependently and specifically with immobilized VWF in an ELISA assay, showing that shear is not necessary to induce the VWF self-association. Whereas anti-VWF monoclonal antibodies (moAbs) had no effect on the self-association, the proteolytic VWF-fragments SpII(1366-2050) and SpIII(1-1365) inhibited the b-VWF-VWF interaction by 70 and 80% respectively. Moreover, a specific binding of b-VWF to immobilized Sp-fragments was demonstrated. Finally, both biotinylated SpII and SpIII were able to bind specifically to both immobilized SpII and SpIII. Similar results were observed under flow conditions, which confirmed the functional relevance of our ELISA system. In conclusion, we have developed an ELISA binding assay in which a specific VWF self-association under static conditions can be demonstrated. Our results suggest a multiple domain interaction between immobilized and soluble VWF.



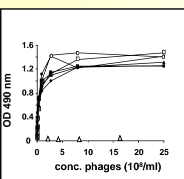
Binding of soluble b-VWF to immobilized VWF under static conditions. Microtiter plates were coated with VWF and incubated subsequently with a dilution series of soluble b-VWF after which bound b-VWF was detected with streptavidin-HRP. (n=3)



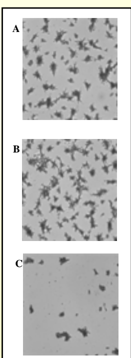
Possible model of VWF self-association. (A) In solution, the globular structure of VWF is maintained by multiple interactions between SpII-SpII, SpIII-SpIII and SpII-SpIII domains. (B) Immobilization of VWF disrupts the multiple domain interactions present in the globular VWF structure. (C) VWF self-association is mediated by the same interactions which are present in globular VWF. Homotypic (i) and heterotypic (ii) domain interactions from the N-and C-terminal portion of VWF modulate VWF self-interaction.

Selection of phages that inhibit von Willebrand Factor interaction with collagen under both static and flow conditions *Thromb. Haemost*, 86, 630-635, 2001 IF 3.351

Phages from a pentadecamer phage display library were selected for binding to VWF by affinity panning. Bound phages were selectively eluted with human collagen type I. After the third round of panning 95 % of individual phage clones bound to VWF. The B8-phage inhibited the binding of collagen to VWF with an IC50 of 0.6 1010 phages/mL, and of VWF to collagen with an IC50 of 1.0 1010 phages/mL at 0.5 µg/mL VWF. Under flow conditions, 1.5 1011 B8-phage/ml nearly completely inhibited platelet deposition on a human collagen type I coated surface at a shear rate of 1200 s-1, while phages without an insert had no effect. The peptide corresponding to the one displayed on the B8-phage competed with the phage for binding to VWF with an IC50 of 30 µg/mL (16 µM). The peptide furthermore inhibited VWF-binding to collagen with a maximum of 40 % at a concentration of 1.25 mg/mL (650 µM), higher concentrations of peptide could not improve this. We thus have selected phages that are potent VWF-binders and that can be used as tools to detect VWF, to inhibit VWF-collagen interaction and to further analyse the role of VWF-collagen binding.



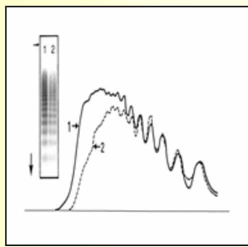
Binding characteristics of phages. a) Binding of different phage clones to microtiterplates coated with 10 mg/mL VWF. ♦ A3, ■ A4, ▲ B3, B6, ○ B7, ● B8-phage and △ an irrelevant phage. The figures are representatives of 3 experiments.



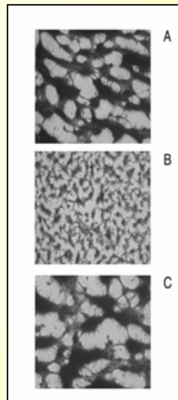
Inhibition of platelet deposition onto a collagen coated surface under flow conditions. Whole blood in the absence or presence of phages ($1.5\ 10^{11}$ phages /mL) was perfused during 5 min over collagen coated coverslips (1200 s-1). A: whole blood, B: whole blood with nonsense phages (without insert), C: whole blood with B8-phages.

A monoclonal antibody directed against human von Willebrand factor induces type 2B-like alterations *J. Thromb. Haemost.* 2: 1622–1628, 2004 IF 4.831

We have previously described a monoclonal antibody (moAb), 1C1E7, against von Willebrand factor (VWF), that increases ristocetin induced platelet aggregation (RIPA) and induces a preferential binding of the high molecular weight multimers of VWF to platelet GPIb. Further investigations using a rotational viscometer at a shear rate of 4000/sec now could demonstrate that shear-induced platelet aggregation (SIPA) is significantly increased with 1C1E7 and that this could be completely inhibited by the anti-GPIb moAb 6D1. In contrast, platelet adhesion to a collagen surface at a shear rate of 2600/sec, using a rectangular perfusion chamber, was significantly inhibited in the presence of 1C1E7. When citrated whole blood was incubated with 1C1E7, a spontaneous binding of VWF to the platelet GPIb could be demonstrated by flow cytometric analysis. Parallel to this, a decrease of the highest molecular weight multimers of VWF in the plasma was found. Platelets with bound VWF on their surface are able to form macroaggregates but are no longer able to adhere. These phenomena are very similar to the alterations described in von Willebrand's disease (VWD) type 2B. The epitope of this moAb could be localized to the N-terminal part of the subunit; therefore a distant conformational change in the A1 domain of VWF is suggested.



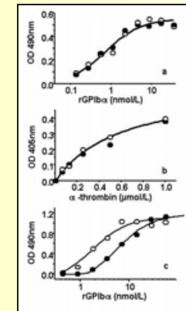
Multimeric analysis of von Willebrand factor in the absence (1) or presence (2) of moAb 1C1E7. Citrated whole blood was incubated in the presence of 20 µg/mL anti-VWF moAb 1C1E7 or 20 µg/mL 82D1E1 (control) for 30 minutes at 20 °C. Then the multimeric structure of VWF was analysed in plasma, using SDS-agarose electrophoresis (insert). The densitometric scan of a representative experiment is shown. The arrows indicate the application site of the samples.



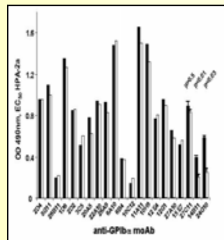
Adhesion of platelets to collagen surface in the presence (A) of PBS (control), in the presence (B) of moAb 1C1E7, in the presence (C) of moAb 23/4B1. Light microscopic picture at 400x magnification. PPACK-anticoagulated whole blood was incubated with either 20 µg/mL 1C1E7, 20 µg/mL 23/4B1 or PBS (control) for 30 minutes at 20°C. Human collagen type III, coated to glass coverslips, was perfused with the blood samples at a shear rate of 2600/sec for 5 minutes at 37°C. Cover slips were fixated with glutaraldehyde and stained with May-Grünwald/Giemsa.

The platelet glycoprotein Ibalpha HPA-2 polymorphism affects VWF binding, but not thrombin interaction. *Arterioscler. Thromb. Vasc. Biol.* 23:1302-1307, 2003 IF 7.432

Glycoprotein (GP) Ibα is the functionally dominant subunit of the platelet GPIb-IX-V receptor complex. The N-terminal domain of the GPIbα chain contains binding sites for a-thrombin and von Willebrand Factor (VWF). The human platelet alloantigen (HPA)-2 polymorphism of the GPIbα gene is associated with a C/T transition at nucleotide 1018, resulting in a Thr/Met dimorphism at residu 145 of GPIba. To study the structural and functional effects of this dimorphism, N-terminal fragments (AA1-289) of the HPA-2a and HPA-2b alloform of GPIbα expressed in CHO cells were used. Of 74 moAbs directed against human GPIbα, two antibodies with an epitope between AA1-59 could differentiate between both alloforms. In addition, VWF bound with a higher affinity to the recombinant HPA-2a fragment or to homozygous HPA-2a platelets. In contrast, no difference was found in the binding of α-thrombin to the recombinant alloform fragments, or of antibodies directed against the α-thrombin binding anionic sulfated tyrosine sequence (AA269-282).
In conclusion, whereas the Thr145Met dimorphism does not affect α-thrombin binding, it does influence the conformation of the N-terminal flanking region and first leucine rich repeat of GPIbα and by this has an effect on VWF-binding.



Ristocetin-induced VWF-binding and a-thrombin binding to rHPA-2a and rHPA-2b. Microtiter plates coated with anti-GPIbα moAb 2D4 were incubated with a dilution series of rHPA-2a (○) or rHPA-2b (●). A 1/32 dilution of plasma in the presence of 1500 µg/mL ristocetin (a) or 760 µg/mL ristocetin (c) was added after which bound VWF was detected. (b) Microtiter plates coated with anti-GPIbα moAb 12G1 were incubated with 1 µg/mL rHPA-2a (○) or rHPA-2b (●). A dilution series of α-thrombin was added. Bound a-thrombin was detected with the chromogenic substrate S-2238. Representative of 4 independent experiments.



Binding of rHPA-2a and rHPA-2b to a panel of 21 anti-GPIbα moAbs.
Binding of rHPA-2a (filled bars) and rHPA-2b (open bars) to 21 anti-GPIbα moAbs coated on microtiter plates was studied. Concentrations shown are the same for rHPA-2a and rHPA-2b and equal to the concentration of rHPA-2a needed for half maximal binding to the respective antibodies (EC50). Bound rHPA-2a or rHPA-2b was detected with b-anti-GPIbα moAb 12G1 and 6B4. For the anti-GPIbα moAbs 27C11, 14B11 and 24G10, mean ± s.e. of 3 independent experiments is shown. For the other anti-GPIbα moAbs, the mean of 2 independent experiments is shown.

Conclusions

- These results enable a better understanding of the molecular interactions between
  1) VWF and collagen
  2) VWF and VWF
  3) VWF and GPIbα
  4) GPIb and thrombin

• this better understanding could lead in the end to the development of more efficient anti-thrombotics

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