The Von Willebrand factor-ADAMTS-13 axis: a two-faced Janus in bleeding and thrombosis

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ABSTRACT

Von Willebrand factor (VWF), a blood multimeric protein with a very high molecular weight, plays a crucial role in the primary hemostasis, the physiological process characterized by the adhesion of blood platelets to the injured vessel wall. Hydrodynamic forces are responsible for the VWF multimers conformational transitions from a globular to a stretched linear conformation. These characteristics render this protein a valuable object to be investigated by mechanochemistry, the biophysical chemistry branch that studies the effects of shear forces on protein conformation. This review will focus on the structural elements of the VWF molecule involved in the biochemical response to shear forces. The stretched VWF conformation favors the interaction with the platelet GpIb and at the same time with ADAMTS-13, the zinc-protease that cleaves VWF in the A2 domain, limiting its prothrombotic capacity. It is important to consider the level or the function of VWF or ADAMTS-13 always in relation each other, keeping in mind that in many thrombotic forms of microangiopathies the reduction of the ratio between the ADAMTS-13 activity and the VWF level (lower than 0.5) can be a valuable parameter to predict a real thrombotic risk. Hence, a significant increase in VWF level alone, even without any reduction of ADAMTS-13 concentration, would still be responsible for a significant reduction of the ADAMTS-13/VWF ratio, which ultimately could reflect or predict a prothrombotic risk. Future studies will have to validate the concept whether ADAMTS-13/VWF ratio could a valuable and reliable biomarker to predict or confirm the presence of thrombotic risk in several morbid conditions.

THE MAIN PLAYERS OF THE GAME: VON WILLEBRAND FACTOR AND ADAMTS-13

Vascular rupture is rapidly counteracted by adhesion and firm attachment of blood platelets at the site of endothelial injury. This process is mostly mediated by Von Willebrand factor (VWF), a multimeric protein of variable molecular weight ranging from about 0.5 to about 20 megadalton, which acts as a bridge between the subendothelial collagen and platelets.[1,2] VWF multimers connect the platelet receptor GpIb/IX/V to collagen fibrils of the extracellular matrix.[2,3] VWF immobilized on subendothelial collagen forms in fact a reactive surface, which captures platelets from flowing blood.[4] Notably, at high shear stress (>30 dyn/cm^2) VWF undergoes micro- and macro-conformational changes from a globular state to a stretched conformation, where especially the A2 domain undergoes relevant structural changes.[5-7] VWF mediates platelet adhesion to the damaged vessel wall under conditions of high shear stress. The VWF gene, localized on chromosome 12, spans 178 kb and contains 52 exons. It is transcribed into an 8.8-kb messenger RNA, which encodes a 2813-aa precursor (pre-pro-VWF) consisting of a 22-aa signal peptide, a 741-aa propeptide and a 2050-aa mature subunit.[1,8,9] The VWF mature subunit is composed of 4 repetitive domains designated A to D and arranged in the sequence D'-D3-A1-A2-A3-D4-C1-C2-C3-C4-C5-C6-CK.5.[1] The platelet GpIbα receptor binding to the A1 domain of immobilized VWF results in an initial adhesion, and a continuous surface translocation of the platelets.[10,11] This process ends with a stable platelet adhesion through interaction with the platelet collagen receptors GpVI and $\alpha_2\beta_1$ integrin (GpIa/IIa),[12-14] activation of the platelet GpIIb/IIIa receptor complex and finally platelet-platelet aggregation. During the process of primary hemostasis, the interaction between VWF and

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GpIbα does not occur under static conditions, but it needs a pre-activation of soluble VWF. As anticipated, this process consists of a conformational change of VWF molecules, which expose within the A1 domain the binding site for GpIbα. This mechanism is triggered either by mechanical forces, such as a high shear rate (>5000 s^{-1}), or shear stress >30 dyn/cm^2, present in the microcirculation. The same effect can also be obtained by chemical potentials generated by the interaction with biochemical effectors, such as the antibiotic glycopeptide ristocetin, the snake venom botrocetin and polyphosphate.[5,6,15-21] However, even natural mutations causing some types 2B von Willebrand disease (VWD), such as the Arg1306Trp, Arg1341Trp, His1268Asp, Arg1306Gln, Arg1308Cys/Leu, Ile309Val, Val1316His, Pro1337Leu, and Arg1341Gln/Trp or in the A1 domain,[22-24] stabilize a stretched conformational state that favors the interaction with the GpIb platelet receptor even under very low shear conditions.[25] However, not all the types 2B VWD variants exhibit the same enhanced interaction with platelets, revealed by the inverse correlation with platelet count.[24] This may derive from the fact that specific amino acid substitutions are probably critical for differently influencing the conformational equilibria and thus type 2B VWD phenotypes. The stretched conformation of VWF not only allows the interaction with the platelet GpIb receptor, but also the proteolytic attack by the zinc-protease ADAMTS-13 (*A Disintegrin And Metalloproteinase with a Thrombospondin type 1 motif, member 13*).[7,25-29] which, cleaving the Tyr1605-Met1606 in the A2 domain of VWF, proteolyzes the molecule and limits the hemostatic and even prothrombotic activity of the ultra large VWF multimers.[7] A shear stress >30 dyn/cm^2 confers the ability to VWF multimers to interact with the platelet GpIb receptor and at the same time to be fragmented by ADAMTS-13 at the Y1605-M1606 peptide bond in the A2 domain. Furthermore, a high shear force favors a VWF self-association, a process by which multimeric VWF binds to or aggregates with additional VWF multimers. This process is responsible for the formation of VWF fibers, that are organized as spider-web like structures.[5,30] This network constitutes an ideal substrate for recruiting flowing platelets in the circulation during the process of primary hemostasis. All these VWF properties stem from the unique capacity of this multimeric protein to be very sensitive to external hydrodynamic forces as many different biological processes,[5] such as cell adhesion, cytoskeleton organization, cell division, bacteria invasion, and the activity of ion channels.[31-37]

The enzyme responsible of the VWF proteolysis is ADAMTS-13. This enzyme is the 13th member of the ADAMTS family of zinc proteases, which is related to the large ADAM (A Disintegrin and Metalloprotease) family. The ADAMTS family of zinc metalloproteases contains 19 members that share the common structure of a hydrophobic signal sequence, a propeptide, a metalloprotease domain (M), a thrombospondin type 1 (TSP1) repeat, a disintegrin-like domain (Dis), a cysteine-rich domain (Cys-R), and a spacer domain (Spa) (Figure 1A).[38] In addition, all ADAMTS family members have one or more thrombospondin type 1 (TSP1) motifs and variable additional C-terminal domains. The carboxyl terminus of ADAMTS-13 contains seven more TSP1 repeats and two CUB domains, which are named after motifs first identified in Complement components C1r and C1s, sea urchin protein uEGF, and Bone morphogenetic protein-1, which can interact with the D4 VWF domain even under static conditions.[39] A recent X-ray diffraction study showed that the ADAMTS-13 CUB1-2 domains have two highly conserved surface patches that form an extended binding site for the central ADAMTS-13 Spacer domain (Figure 1B).[40] The interaction of the CUB1-2 domains with the Spacer domain regulates the ADAMTS-13 global latency by locking ADAMTS-13 in a closed conformational status, which is only released, physiologically, when ADAMTS-13 interacts with VWF.[40,41] Thus, not only VWF undergoes extensive conformational transition under the effect of high shear stress, but even ADAMTS-13 is subjected to allosteric transitions upon its interaction with VWF, resembling a sort of sequential model of allosteric behavior.[42] Experimental steady-state enzymatic studies, which showed a hyperbolic mixed-type inhibition of the hydrolysis by ADAMTS-13 of a VWF peptide substrate,[28,29] also confirmed this model.[43,44] In this review, the relationship between the ADAMTS-13 and VWF levels (ADAMTS-13/VWF ratio) will be discussed concerning its potential role as a biomarker of thrombotic and vascular disorders.

MECHANISMS OF SHEAR-INDUCED REGULATION OF THE ADAMTS-13/VWF INTERACTION

The high sensitivity of VWF to hydrodynamic forces raises the question concerning the molecular mechanisms by which VWF multimers undergo major conformational changes responsible for the acquisition of their full prohemostatic and even prothrombotic activities. The interaction of VWF multimers with cell receptors and other proteins were investigated by using the principles of mechanochemistry.[45] The principles of mechanochemistry find a proper application in biological systems, where macromolecules in living systems do not often interact each other freely in solution, according to ideal kinetics and thermodynamics principles, but frequently interact tethered to other subcellular or matrix components and in a highly structured mode. To characterize the acting biophysical mechanisms, different types of bonds were iden-

tified, such as the *catch-slip bond,*[46-48] and *flex bond.*[49] These binding models assume that the interaction between two macromolecules depends on external forces that exponentially accelerate the dissociation between interacting particles or *ideal bond*,[50,51] indicating that the bond lifetime is independent of an externally applied force. Notably, the *flex bond* was demonstrated to occur in the binding of the VWF A1 domain to GpIb.[49] By using the ReaLiSM method (repeated measurements of the binding and unbinding of a receptor and ligand in a single molecule), Kim et al. showed the existence of two states of the receptor-ligand bond, that is, a *flex-bond.*[49] One state was observed at low hydrodynamic force (<5 pN), whereas a second state begins to engage at about 10 pN with a □20-fold longer lifetime and higher force resistance. The second state can resist to flowing forces that could disaggregate the platelet plug. Although many experimental evidences were provided by different authors, no definitive conclusion has been reached on what type of bond stabilizes and/or controls VWF interaction with platelets or the vessel wall.[45] It must be remarked that the peculiarity of VWF interaction with cell receptors or other proteins resides in its biochemical plasticity, which allows the protein to follow *ideal* or *mechanochemical* pathways in performing its biological functions, as described below.

THE EFFECT OF SHEAR FORCES ON VON WILLEBRAND FACTOR IN DISEASE AND NORMAL CONDITIONS

Although most of the interactions that VWF can make with many macromolecular ligands are possible only if VWF assumes a stretched conformation under high shear forces, the interaction with subendothelial collagen or other molecules can take place even by globular VWF conformer, as collagen was demonstrated to be rather insensitive to hydrodynamic forces.[52-54] Collagen III plays the most relevant role in the interaction with the VWF A3 domain, whereas type VI collagen has only an ancillary role, useful in the case of natural mutations that impair the A1 interaction with type III collagen.[52] As anticipated above, the in-

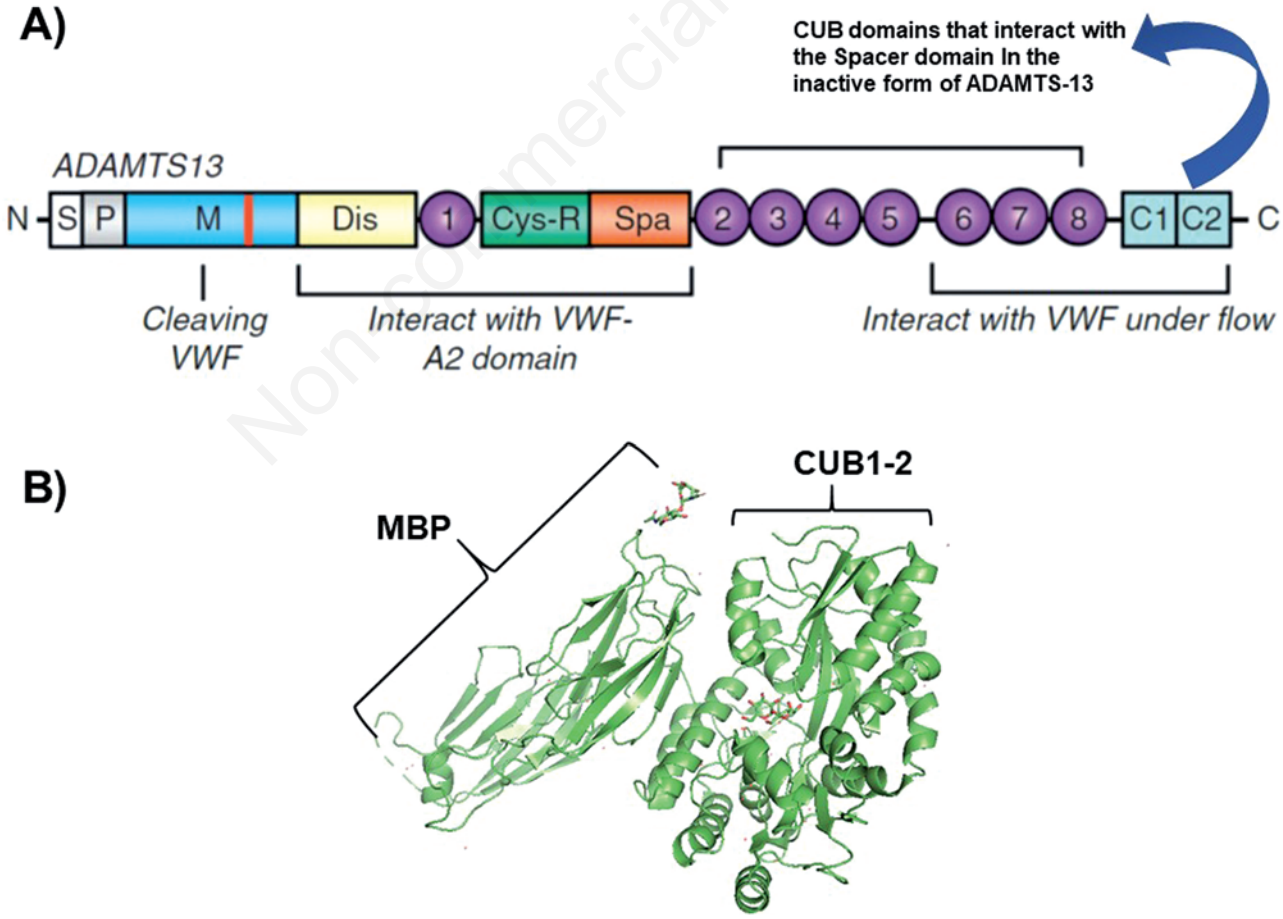

Figure 1. A) Cartoon showing the global structure of the ADAMTS-13 structure. B) Crystal structure of the CUB1-2 conjugated to maltose-binding protein (MBP) (PDB code «7B01») (40). The image rendering was performed with the PyMOL program.

teraction between the VWF A1 domain and the GpIb/IX/V platelet receptor can occur only in the microcirculation, where a shear rate >2000 sec^{-1} is generated. From the mechanochemistry standpoint, the interaction between VWF and collagen can be characterized by an "*ideal bond*", independently from external applied forces. This finding agrees with the possibility to measure the interaction between HMW-VWF with collagen in ELISA tests even under static conditions in the common diagnostic practice of von Willebrand disease (VWD).[55] Another type of *ideal bond* interaction concerns the VWF binding to the low-density lipoprotein receptor-related protein-4 (LRP4), a newly identified receptor for VWF on smooth muscle cell of vessel wall.[56] This interaction seems to play a central role in the vessel intima hyperplasia, favouring ischemic diseases. VWF multimers bound to subendothelial collagen form a reactive surface capable of capturing platelets from circulating blood.[57,58] Furthermore, when VWF multimers are bound to collagen, their rotational entropy, present when the protein is free in solution, is drastically reduced and the elongational force becomes predominant.

THE ROLE OF SHEAR FORCES IN DIFFERENT FORMS OF VON WILLEBRAND DISEASE

The shear force acts on the multimers so that the latter form an elongated strain on the endothelial surface that exposes multiple binding sites for ligands and receptor. Finally, even gain-of-function natural mutations causing type 2B VWD, such as the p.R1306W or p.R1341W, are able to stabilize a conformational state that is prone to the interaction with the platelet receptor GpIb even under very low shear conditions, thus causing platelet clumping in the systemic circulation.[23,59] As anticipated above, a shear stress level >30 dyn cm^2 would cause the unfurling of VWF multimeric strings. This stretching phenomenon can expose the A1 domain that binds to the platelet GpIb. However, under certain shear stress conditions the macroconformational transition is accompanied by a degree of molecular flexibility that allows the VWF A1 residues engaged in the binding to GpIb to fit into the interacting cleft of the receptor.[60] Natural mutations causing type 2M VWD, such as p.G1324A and p.G1324S, generate a more rigid sequence due to the substitution of the simple side chain of glycine (hydrogen atom) with a larger side chain (methyl group and hydroxyl group of alanine and serine, respectively) that strongly limits the conformational flexibility of that part of the molecule contained in the β-turn between β-strands 2 and 3 within the native state of VWF.[60] Hence, not even external hydrodynamic forces are able to counteract the rigidity of this segment of the protein, which has become much more rigid due to the type 2M mutations. By contrast, in the wild type VWF hydrodynamic forces induce conformational transitions that, occurring mostly in the A2 domain, propagate thereafter through the A1 and other domains of the monomer, causing ultimately a stretching of the multimers.

THE A2 DOMAIN OF VWF IS A MECHANOSENSITIVE SWITCH

The X-ray crystal structure of the A2 domain showed that it resembles the overall conformation of the so-called VWA domain,[6] present in a protein superfamily comprising integrins on cell surfaces, complement components and DNA repair proteins.[61] However, the A2 domain shows a unique structure if compared to other VWA domains. In fact, the A2 domain has a sequence of α-helices and β-strands that alternate in sequence (Figure 2). The hydrodynamic shear forces act mostly on this domain, as the unfolding will proceed from the C-terminal end rather than the N-terminal end of the A2 domain, because the C-terminal structural elements can be more easily moved one at a time from the end of the domain, while the β1 strand at the N-terminal is surrounded by counterforts constituted by β2 and β4 strands in the core of the domain (Figure 2). Based on this structural arrangement, any external hydrodynamic force acting on the C-terminus of the A2 domain will be simultaneously sensed by the 8-membered Cys1670-Cys1669 disulfide ring, which acts a high energy barrier to

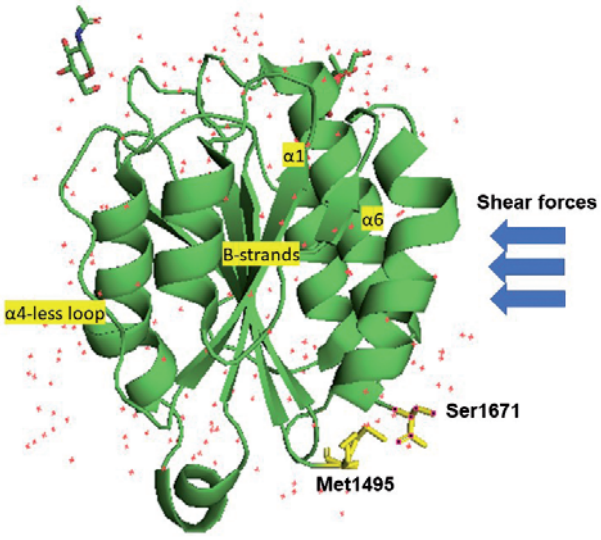

Figure 2. Ribbon diagram of VWF A2 domain of human A2 domain (PDB code "3GXB") (6). The red cross represents the hydration water molecule. Note the presence of several helices at the lateral sides of the molecule and the b-strands at the mid of the molecule. The shear forces push from the C-terminus toward the N-terminal of the molecule to induce the stretching of the domain with the exposition of the Tyr1605-Met1606 peptide bond cleaved by ADAMTS-13. The image rendering was performed with the PyMOL program.

the external force. Once this energetic barrier is overcome, the external force will be transmitted to the hydrophobic core containing the Tyr1605-Met1606, because of the apolar interactions with Leu1603 and Tyr1605. At this point, the poor packing of the β4 strand and the high conformational flexibility of the long loop replacing the α4 strand give rise to an easy unfolding of the hydrophobic core. The unfolding of the inner hydrophobic core of the A2 domain is accompanied by solvation of the region and the Tyr1605-Met1606 becomes available to the proteolysis by ADAMTS-13. The structural peculiarity of the vicinal disulfide Cys1669-Cys1670 plays a central role in the mechanosensitive properties of the entire A2 domain, whose genetic mutations are largely responsible for many forms of type 2A von Willebrand disease (p.R1597W, p.G1505E, p.I1628T, and p.E1638K) that shows a loss of high molecular weight multimer because of an increased proteolysis by ADAMTS-13 in the bloodstream. The supernormal proteolysis by ADAMTS-13 is linked to the tendency of these VWF mutants to be in a more "open", unfolded conformations that render the Y1605-M1605 in the hydrophobic core of the β4 strand susceptible to the proteolytic attack by the metalloprotease. The activity of high shear forces triggers a shift toward the unfolded form of the A2 domain, as unraveled by the X-ray diffraction studies by Zhang *et al*.[6] It must be outlined that in the flowing blood VWF multimers are exposed to high hydrodynamic forces for a limited time and, due to the reversibility of the unfolding reaction, ADAMTS-13 could exert its proteolytic activity for a very short time.[28,29] However, the nature has provided VWF with a biochemical mechanism that allows conversion from a cis- to a trans W1644-P1645 peptide bond.[62] This structural transition is capable to greatly delay the process of refolding of the A2 domain, thus allowing a more prolonged activity by ADAMTS-13. Once the unfolding of the A2 domain takes place, conformational transitions involve also the vicinal A1 and A3 domains, so that the binding sites for other ligands become available for interactions. Hence, the conformational instability of the A2 domain under sufficient shear forces can also affect the interaction between the A1 and A2 domains. These two domains are adjacent to each other and connected by a linker of 30 amino acids. Under low shear conditions, the A1 domain, and particularly the GpIb binding site, is shielded by a specific inhibitory interaction with the vicinal A2 domain.[53] Thus, once enough shear force overcomes the resistance of the A2 domain to unfold, the conformational transition in the A2 domain gives rise to two different effects: 1) the exposition of the Y1605-M1606 to solvent, rendering it available to the proteolytic attack by ADAMTS-13; 2) the elimination of the shielding effect of the A2 on the A1 domain with exposition of the GpIb binding site in the A1 domain. Furthermore, the shear force-dependent stretching of the whole VWF multimer creates many binding sites available for interactions with cell receptors and other protein ligands.

THE ROLE OF THE MOLECULAR DIMENSION OF VWF MULTIMERS IN SHEAR-INDUCED STRETCHING

The unfolding of A2 domains, occurring under a ≈11 pN tensile force,[6] and its hydrolysis of by ADAMTS-13, occurs preferentially near the center of untangled multimers. The ultra large VWF multimers undergo rapid scission upon secretion into the vasculature because of their length.[63] In fact, the tensile force F(j) to the inside of any sphere pair j in a chain with N dimers, is the sum of the force on all the outer dimer pairs. Hence, the total tensile force is given by[7,64]

$$F(j) \approx \sum_{i=j}^{N} f[i\,x\,(d+2a)] \approx \frac{(N+j)(N+1-j)}{2} f(d+2a)$$

Where *f(i)* is the normal force between two spheres that are a certain distance (*x*) apart, *d* is the length of the rigid tether between two monomers, and *a* is the radius of the monomer. From eq. 1, the normal force on a monomer in the center of a multimer is roughly proportional to N^2 (*i.e*., when j=1), whereas the force on a monomer at the end of a multimer is proportional to N (*i.e*., when j = N). This quadratic dependence markedly separates longer and shorter multimers, if referred to the capacity of external shear force to stretch the molecule. Zhang *et al*. calculated that 11 pN can unfold an upper length limit for VWF of 200 monomers, as observed in atomic force experiments.[7] Thus, it should be outlined that the length of VWF multimers is essential for their hemostatic function: short multimers are not able to reach sufficient forces to induce the conformational transition described above. Another advantage of having sufficiently long multimers resides in their ability to expose more interaction sites than shorter chains. In other words, the stretching mechanism can increase the stoichiometry of VWF/ligand interaction, comprising ADAMTS-13. The occurrence of these biophysical mechanisms finds its expression in pathological conditions responsible for opposite effects. Inherited VWF diseases characterized by severe deficiency of high molecular weight VWF multimers (*e.g.* type 2A VWD) cause hemorrhages, whereas in the acquired disease referred to as thrombotic thrombocytopenic purpura, the accumulation of ultra-large VWF multimers, due to deficiency of ADAMTS-13, causes thrombotic complications in the microcirculation, where high shear stress is generated.[65-67] Natural mutations of the VWF A1 domain may differently affect the conformational transitions of the whole VWF multimer linked to external hydrodynamic forces, as noticed above for the type 2M von Willebrand mutations

p.G1324A and p.G1324S, as described above. This effect is associated with resistance to unfolding induced by denaturing agents, reduced sensitivity to hydrodynamic force and decrease of affinity for GpIb under shear flow and resistance to limited proteolysis.[60] By contrast, the natural mutation R1306W in the A1 domain, causing a type 2B von Willebrand disease, switches the equilibrium toward an unfolded conformation of VWF multimers, thus showing a higher sensitivity to shear stress,[23] which facilitates exposure of GPIb binding sites. The mean hydrodynamic diameter of resting p.R1306W VWF multimers was indeed significantly greater than that of wild type VWF multimers (210±60 nm *vs*. 87±22 nm, respectively).[60] At shear forces <14 dyn cm^2, the p.R1306W multimers rapidly populates stretched conformers, acquiring the capacity to interact with GpIb which, instead, was induced for WT VWF by shear forces >30 dyn cm^2 only.[60]

Another relevant VWF activity gained upon attainment of a stretched conformation under appropriate shear forces is the propensity to self-aggregate.[5] Thus, when the unfolding prevails under the effect of shear forces, VWF multimers act as promoters of thrombotic mechanisms but at the same time are available to the proteolysis by ADAMTS-13. When this proteolytic process cannot take place, due to the formation of autoantibodies against ADAMTS-13 or for genetic deficiency of the metalloprotease, thrombotic microangiopathy occurs, characterized by an accumulation of ultra-large VWF multimers.[66,68] Upon stabilization of the stretched conformation, the swapped domains of a VWF multimer can interact with the same domain but belonging to a different multimer. Hence, the non-covalent intra-monomer interactions between domains are broken and restored between different multimer chains: this aggregation mechanism may be an example of the biophysical phenomenon referred to as *3D domain swapping*.[69,70] The latter is a mechanism that allows protein molecules to form dimers or polymers by exchanging identical domains. More properly, we should consider the process of VWF multimerization and subsequent molecular aggregation as a *3D domain swapping* mechanism.

THE ADAMTS-13/VWF CONCENTRATION RATIO: A DELICATE BALANCE BETWEEN HEMOSTASIS AND THROMBOSIS

The kinetics of the interaction between the zinc-protease ADAMTS-13 and its substrate VWF has been extensively investigated.[28,29,71] Under "physiological" conditions of temperature, pH, and calcium/zinc/chloride ion concentration, under steady state conditions ADAMTS-13 cleaves VWF at the Tyr1605-Met1606 peptide bond with a K_m in the low µM range, with a k_{cat} of about 1-2 sec^{-1}, and a k_{cat}/K_m of ≈5 x10^5 M^{-1} sec^{-1}.[28,29] These parameters were obtained using in vitro the Michaelis-Menten approximations considering steady state conditions, being the concentration of the substrate VWF>ADAMTS-13, as found in blood in physiological conditions (ADAMTS-13: 3-4 nM; VWF:Ag: ≈40 nM). However, as anticipated in the preceding paragraph the interaction between ADAMTS-13 and VWF shows a particular cooperativity, being characterized by a *hyperbolic mixed-type inhibition by product*.[28,29] Hence, the higher the ultra-large VWF concentration the higher is the inhibition mechanism by its substrate (Figure 3). Under certain clinical conditions (inflammatory status, cancer, cardiovascular diseases, viral infections, etc.) where a massive secretion of VWF may occur, the excess of the substrate causes a functional insufficiency of ADAMTS-13 to proteolyze ultra-large VWF with a progressive accumulation of these multimers. These conditions are characterized by a marked decrease of the ADAMTS-13/VWF ratio <0.5 can be considered a biomarker of enhanced risk of thrombotic disorders.[72]

THE RELEVANCE OF THE ADAMTS-13/VWF RATIO IN ISCHEMIC/THROMBOTIC AND HEMORRHAGIC DISORDERS

The relative levels of both ADAMTS-13 and VWF in healthy subjects can be described by a ratio close to unity (1.0), although it may range from about 0.5 to 2.0. When

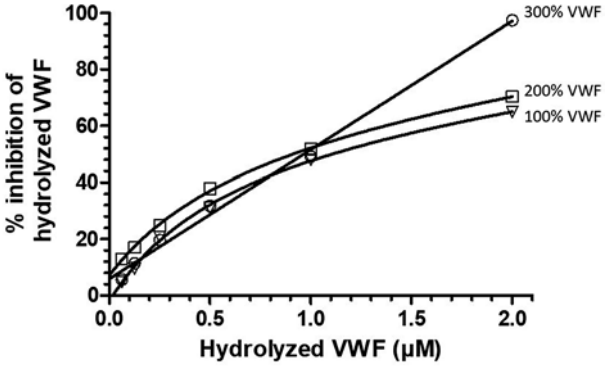

Figure 3. Simulation effect of the hyperbolic mixed-type inhibition by hydrolyzed VWF (product) on the inhibition degree of the hydrolysis of VWF as substrate at the indicated concentration. The points were calculated by the appropriate parameters of the hyperbolic mixed-type inhibition equation, as previously reported: $V_i = k_{cat} * ESZ°/[(KmZ^1) + (SZ^2)]$, where $Z° = 1+(\beta I/\alpha K_i)$; $Z^1 = 1 + (I/K_i)$; $Z^2 = 1 + I/\alpha K_i$) and K_m of VWF cleavage by ADAMTS-13 equal to 1 µM, k_{cat}=1sec^{-1}, K_i is the equilibrium dissociation constant of cleaved product binding to ADAMTS-13= 1 µM, a=10 (the corrective factor for K_i when the product binds to the ES complex), and b=0.23 (the corrective factor for k_{cat} concerning the ESI adduct) (18). VWF:Ag level 100%=10 µg/ml≈40 nM. The calculations were performed with the GraphPad Prism software.

this ratio falls in this range the VWF/ADAMTS-13 axis is balanced. In VWD, the ADAMTS-13/VWF ratio>>2.0 or approaching infinity in the case of type 3 VWD. At variance with congenital hemorrhagic disorders, in thrombotic microangiopathies, such as acquired or congenital severe Thrombotic Thrombocytopenic Purpura (TTP) the ADAMTS-13/VWF ratio approaches zero. The ADAMTS-13/VWF ratio has also been reported to have clinical significance in several ischemic/thrombotic conditions. For instance, as a predictor of outcome in acute ischemic brain injury,[73,74] predicting thrombotic complications in cirrhosis and post-hepatectomy,[75-77] and as a prognostic factor after acute myocardial infarction or infection by Sars-CoV-2.[78-81] In these vascular conditions the scenario is dominated by an increase of ultra-large VWF multimers. This occurs under the pressure of inflammatory cytokines or other secretagogues substances (adrenergic molecule, thrombin, FXa, etc.) that facilitate the secretion of Weibel-Palade granules containing the ultra-large forms of VWF. If in this situation the ADAMTS-13 level remains constant or even decreases, the physiological stoichiometry ratio between ADAMTS-13 and VWF is significantly reduced, favoring the presence in the circulation of the ultra-large forms of VWF. Clinical trials are underway to demonstrate the pathogenetic role of a reduced ADAMTS-13/VWF ratio in various cardiovascular and thrombotic diseases and its use as a prognostic biomarker of the outcome. At variance with the above settings, in other clinical conditions, such as those found in subjects with type O blood group, patients with some type 2A VWF variants, or bearing ventricular assisted devices, the interaction between ADAMTS-13 and VWF multimers is facilitated, due to altered VWF conformation. This effect induces the exposition to solvent of the Tyr1605-Met1606 peptide bond in the A2 domain that favors the proteolytic attack by ADAMTS-13,[82-84] causing the loss of high molecular weight VWF multimers and hemorrhagic diathesis. Table 1 summarizes the different conditions, in which the perturbation of the ADAMTS-13/VWF ratio is associated with thrombotic and hemorrhagic phenotypes.

WHAT ABOUT THE ROLE OF THE ADAMTS-13/VWF AXIS IN EXTRA-HEMOSTATIC FUNCTIONS OF VWF AND THE METALLOPROTEASE?

It has definitively been established that the biological roles of VWF and ADAMTS-13 are not limited to hemostasis, as they are also engaged in other processes, such as angiogenesis and cell proliferation.[85-89] VWF also undergoes a secretion through the basal pole of the endothelial cells, thus being present also in the subendothelial space. A very recent study has demonstrated that basal secretion by endothelial cells of Weibel-Palade bodies causes subendothelial and abluminal VWF retention that stimulates smooth muscle cell (SMC) proliferation through the activation of low-density lipoprotein receptor-related protein-4 (LRP4) in cooperation with integrin aVb3 by the A2 domain of VWF.[56] It is still unknown whether ADAMTS-13 is present in the subendothelial spaces and whether can proteolytically process VWF in this extravascular milieu. However, the available preliminary data pave the way to a wider comprehension of many biological functions performed by VWF and ADAMTS-13. Whether the ADAMTS-13/VWF ratio plays a significant role even in these extra-hemostatic functions of the proteins is not yet discovered, although we can hypothesize that this is the case.

CONCLUSIONS

In this review, we have described how the high sensitivity of VWF multimers to mechanical forces, which is centered in the A2 domain, plays a pivotal role in the pathophysiology of several hemorrhagic and thrombotic disorders. However, we should always keep in mind that when we consider the level or the function of VWF or ADAMTS13 alone, without considering the other partner, this can lead to a limited interpretation of clinical or experimental findings. Hence, a significant increase in VWF level

Table 1. Abnormal ADAMTS-13/VWF ratio in various thrombotic and hemorrhagic disorders.

ADAMTS-13/VWF ratio	Clinical condition	VWF pattern	Symptom	References
<<0.50	Acquired TTP	High molecular weight multimers ↑	Microcirculatory thrombosis	(66, 68)
<0.50	Acute hepatic failure with portal vein thrombosis. Cerebral ischemia. Endothelial dysfunction in viral infection.	High molecular weight multimers ↑	Thrombosis	(73, 75-77, 79-81, 90, 91)
≅1 or >1 but with enhanced VWF susceptibility to cleavage by ADAMTS-13	Blood group 0. Type 2A VWD. VAD-bearing patients.	High molecular weight multimers ↓	Hemorrhage	(82-84)

VAD, Left Ventricular-Assisted Device.

alone, even without any reduction of ADAMTS13 concentration, would still be responsible for a significant reduction of the ADAMTS-13/VWF ratio and could ultimately reflect or predict a prothrombotic risk. Further studies must be conducted to validate the concept whether ADAMTS-13/VWF ratio could a valuable and reliable biomarker to predict or confirm the presence of prothrombotic conditions.

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