How to keep the factor VIII/von Willebrand factor complex in the circulation

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von Willebrand disease and hemophilia A: current treatments

von Willebrand factor (VWF) and factor VIII (FVIII) make an enigmatic duo that is present in the circulation as a tightly bound complex.[1] Their individual roles in hemostasis have been well-established, and functional deficiency of either the former or the latter protein is associated with severe bleeding complications, known as von Willebrand disease (VWD) and hemophilia A, respectively. Over the last several decades, the clinical management of the severe forms of these disorders predominantly relied on replacement therapy using concentrates enriched in VWF, FVIII or both. In contrast, the moderate and mild variants of VWD and hemophilia A benefited from using desmopressin, a vasopressin 2-receptor agonist that stimulates the rapid release of endothelial VWF and FVIII.[2,3] Despite its numerous advantages (such as ease of administration, low costs, no risk of inhibitor development or of transmittable disease), desmopressin also has a number of limitations. Post-treatment increases of VWF and FVIII are transient and limited by the natural short half-life (about 12 h) of the FVIII/VWF complex, and repetitive use of desmopressin results in a diminished responsiveness (tachyphylaxis) due to exhaustion of the VWF storage organelles.[2,4] Furthermore, desmopressin has variable effectiveness in VWD-type 2A and 2M as well as in hemophilia A, and it is contraindicated for VWD-type 2B as it may worsen the thrombocytopenia in these patients.[2,5] It is worth noting that desmopressin is foremost an anti-diuretic, and the desmopressin-induced secretion of VWF from storage organelles is actually an off-target effect. Finally, desmopressin use is associated with some side effects (transient headaches, facial flushing, hypotension, hyponatremia and mild tachycardia), although these are generally mild and well-tolerated.[6,7]

Increasing endogenous von Willebrand factor and factor VIII levels

While the abovementioned treatment options are satisfactory to some extent, treatment still needs to be optimized. With regard to severe hemophilia A, new approaches have been approved for the clinic (e.g., extended half-life variants of FVIII, emicizumab) or are in advanced clinical development (e.g., fitusiran, concizumab, marstacimab, efanesoctocog alfa, valoctocogene roxaparvovec). In contrast, few novel strategies are emerging or even appearing on the visible horizon with regard to VWD or mild hemophilia A. Interestingly, the majority of patients with VWD or mild/moderate hemophilia A could already benefit from an increase in endogenous levels of the VWF/FVIII complex, as is evident from the successful use of desmopressin. It could thus be worthwhile designing approaches that aim to increase endogenous FVIII and VWF levels in a more sustainable manner compared to desmopressin. A first approach was described already 20 years ago: treatment with interleukin-11 was associated with an increase in VWF in both mouse and canine models.[8,9] The underlying mechanism seemed to be related to an upregulation of VWF mRNA in response to interleukin-11.[9] However, follow-up phase II clinical studies were somewhat disappointing, as treatment with interleukin-11 was associated with only a modest rise in VWF plasma levels (1.1-1.5 fold).[10,11]

The anti-von Willebrand factor aptamer BT200

In this issue of *Haematologica*, Kovacevic and colleagues present a new strategy that is associated with increased VWF/FVIII levels, centered around the aptamer BT200.12 Originally, the authors developed BT200 as an antithrombotic agent to interfere with the platelet-binding activity of VWF.[13] BT200 is a short hairpin-structured oligonucleotide consisting of the methylated nucleobases adenine, cytosine, guanine and uracil, and is an optimized derivative from the previously described aptamer ARC1779.[14] Both BT200 and ARC1779 have in common that they specifically bind to the A1 domain of VWF, thereby interfering with the binding of VWF to its platelet-receptor glycoprotein Ibα (GpIbα).[13] Preclinical studies in primates demonstrated that the improved BT200 aptamer is not only highly efficient (inhibition of VWF A1 domain activity: IC_{50} = 70-180 nM), but also has an excellent bioavailability following subcutaneous injection (>77%) and a long half-life (>100 h) due to its pegylated character.[13] Studies using blood samples of stroke patients further confirmed that BT200 has a potent antithrombotic activity.[15]

Initial studies using ARC1779 revealed that this molecule led to an increase of VWF levels in patients with VWD-

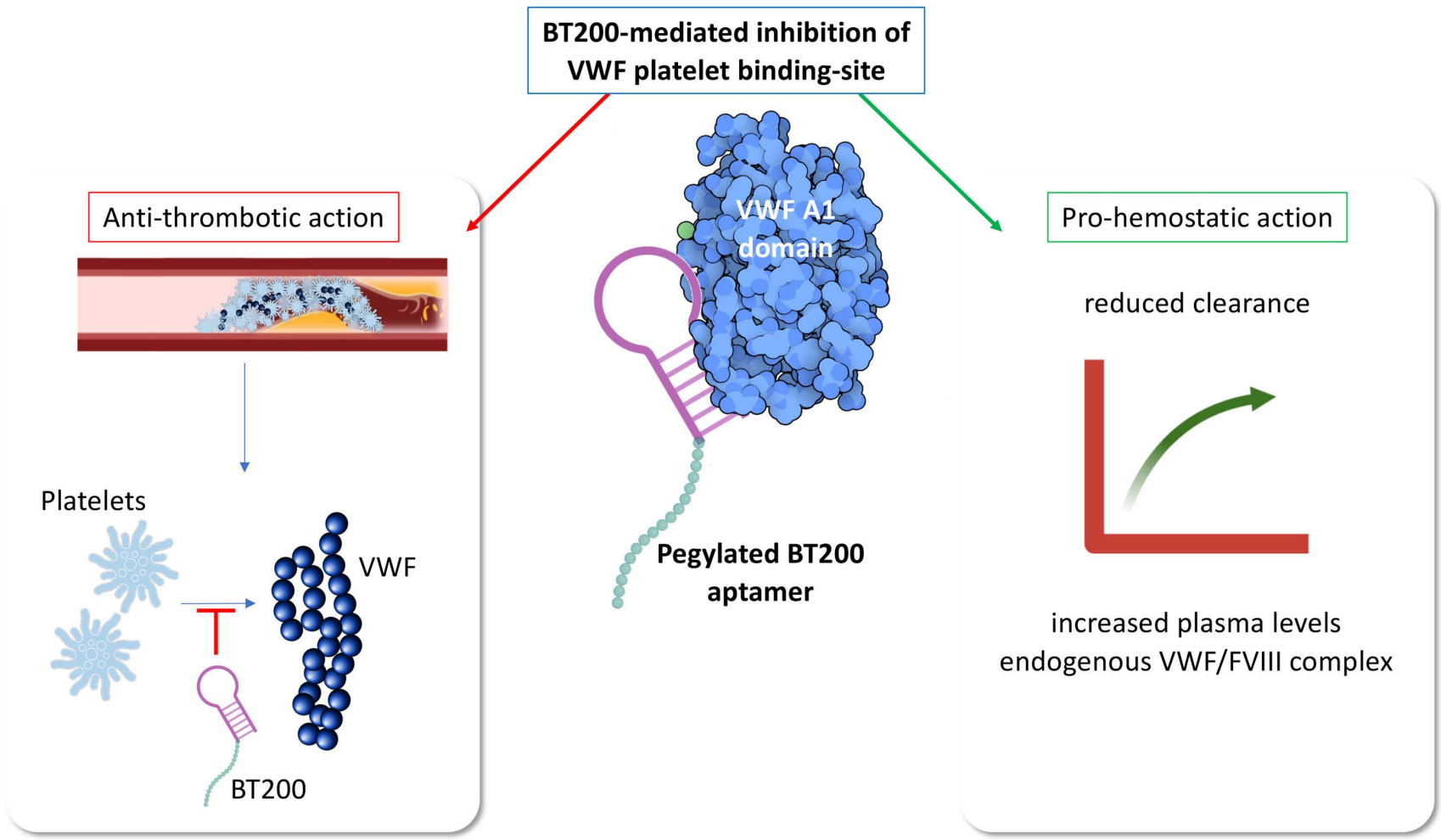

Figure 1. Mode of action of BT200. BT200 is a pegylated aptamer that binds with high affinity to the platelet-binding site within the von Willebrand factor (VWF) A1 domain. It was previously shown that BT200 interferes with VWF-dependent thrombus formation, which endows the molecule with efficient antithrombotic activity.[13] In their study, Kovacevic and colleagues now demonstrate that BT200 also delays clearance of the VWF/factor VIII (FVIII) complex, resulting in transient increases in plasma levels of both proteins. This approach could thus be a strategy to increase endogenous VWF/FVIII levels in patients with mild/moderate forms of von Willebrand disease and hemophilia A.

type 2B.[16] These results prompted the investigators to initiate a larger first-in-human prospective phase I study using the improved BT200 variant, the outcomes of which are reported in this issue of *Haematologica*.[12] In a single-dose bioavailability study, participants received 0.18 to 36 mg BT200 subcutaneously. This resulted in a dose-dependent increase in BT200 plasma concentrations, with maximal levels being around 3 μg/mL after 168 h when the highest dose was given. Increasing doses were associated with a dose-dependent occupation of the VWF A1 domain, with 75%-90% of A1 domains being occupied at doses between 12 and 24 mg. Maximal occupation was observed between 1 and 4 days after injection, after which free A1 domains gradually re-appeared and returned to normal levels 2 weeks after the injection. Interestingly, the administration of BT200 at these doses was associated with 3- to 4-fold increases in VWF antigen levels. However, VWF antigen levels peaked between 7 and 14 days after injection, somewhat later compared to maximal occupation of the A1 domains. Concurrent to the increase of VWF antigen, there was also a 2.5-fold increase in FVIII activity. Of note, FVIII levels could be further increased via the administration of desmopressin, indicating that the mechanisms by which FVIII levels are increased by BT200 and desmopressin are different. Indeed, BT200 appears to act by prolonging the half-life of VWF rather than modifying its synthesis or secretion. As such, its mode of action is fundamentally different from that of desmopressin or interleukin-11.

In view of this listing of impressive data, it seems conceivable that BT200 is an attractive candidate to ameliorate endogenous levels of the FVWF/FVIII complex. Of course, these data are derived from an initial phase I study, and several issues would require additional investigations. For instance, BT200 is designed to interfere with VWF activity, and data presented in the *Online Supplementary Material* show that doses inducing the highest increase in VWF/FVIII levels were also associated with prolonged closure times in the platelet function analyzer assay and provoked reduced platelet aggregation activity, at least during (part of) the first week.[12] It is therefore going to be key to find the optimal dosing that allows increased FVIII and VWF levels, without compromising the patient's hemostatic potential. It should be noted that in the case that the activity of BT200 needs to be neutralized, the authors have already developed an efficient reversal agent, i.e., a complementary aptamer designated BT101, which specifically binds BT200 with high affinity.[17]

It is interesting to speculate further on the clinical ap-

plication of this molecule: designed as an antithrombotic agent, which could be used for VWF-dependent thrombotic complications (arterial thrombosis, thrombotic thrombocytopenic purpura); it may now also find a use in the treatment of bleeding disorders such as mild/moderate hemophilia A and certain types of VWD (Figure 1). It is unusual to find both features in a single molecule!

In conclusion, Kovacevic *et al.* present an elegant approach to improve endogenous VWF and FVIII levels through a single subcutaneous administration of BT200. We look forward to seeing additional clinical data.

Disclosures

No conflicts of interest to disclose.

Contributions

CVD and PJL wrote the manuscript.

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