Multimeric Composition of Factor VIII/von Willebrand Factor Following Administration of DDAVP: Implications for Pathophysiology and Therapy of von Willebrand’s Disease Subtypes

By Zaverio M. Ruggeri, Pier Mannuccio Mannucci, Rossana Lombardi, Augusto B. Federici, and Theodore S. Zimmerman

We have studied the modifications in the multimeric composition of plasma factor VIII/von Willebrand factor and the bleeding time response following administration of 1-Deamino-[8-D-Arginine]-Vasopressin (DDAVP) to patients with different subtypes of von Willebrand’s disease. In type I, all multimers were present in plasma in the resting state, though they were decreased in concentration. Administration of DDAVP resulted in an increased concentration of these forms as well as the appearance of larger forms than were previously present. There was concomitant correction of the bleeding time. In type IIA, large multimers were absent in the resting state, and although DDAVP induced an average threefold increase in the plasma concentration of factor VIII/von Willebrand factor, the larger multimers did not appear and the bleeding time, although shortened, was not corrected. In contrast, the larger multimers that were also absent from type IIb plasma in the resting state rapidly appeared following DDAVP administration. However, their appearance was transitory and the bleeding time, as in IIA patients, was shortened but not corrected. The characteristic multimeric composition of platelet factor VIII/von Willebrand factor in given subtypes predicted the alteration in plasma factor VIII/von Willebrand factor induced by DDAVP. These studies provide evidence that the different subtypes of von Willebrand’s disease represent distinct abnormalities of factor VIII/von Willebrand factor. They also suggest that complete hemostatic correction following DDAVP can be routinely expected only in type I von Willebrand’s disease, and only if factor VIII/von Willebrand factor can be raised to normal levels.

It has been shown that 1-Deamino-[8-D-Arginine]-Vasopressin (DDAVP) increases the plasma levels of factor VIII/von Willebrand factor (FVIII/vWF) presumably by release from tissue sites. This property of DDAVP has been utilized successfully in the treatment of milder forms of hemophilia A and von Willebrand’s disease.2-10 The potential advantages of this synthetic drug over plasma products are numerous. They include low cost and prompt availability, lack of exposure of patients to infusion of plasma proteins, and freedom from viral hepatitis. However, DDAVP has not been uniformly effective in correcting the abnormal hemostasis of patients with von Willebrand’s disease. It seemed likely to us that this was related to individual differences in multimeric composition and functional properties of FVIII/vWF.11-20 We have therefore studied the multimeric composition of plasma FVIII/vWF and the bleeding time response following administration of DDAVP to individuals with different subtypes of von Willebrand’s disease (Table 1). The results obtained provide insight into the distinct pathophysiologic mechanisms underlying these subtypes. In addition, they indicate why the hemostatic response to DDAVP can be expected to vary from subtype to subtype.

MATERIALS AND METHODS

Patients and Controls

The patients described in this study had von Willebrand’s disease type I (five cases), type IIA (six cases), and type IIb (five cases) according to a previously published classification16 (Table 1) and have been fully described elsewhere.17-21 Normal controls were three male and three female healthy volunteers from the Policlinico Hospital staff. All subjects were aware of the experimental nature of the studies and gave their informed consent, and all experiments were performed according to the Declaration of Helsinki. DDAVP (trademark: Minirin; Valeas, Milan) is licensed for use in the treatment of von Willebrand’s disease in Italy, where all experiments were performed.

Methods

DDAVP, at the dose of 0.4 μg/kg body weight, was diluted into 100 ml of normal saline and infused i.v. over a 30-min period. Blood pressure and pulse rate were monitored during the infusion and no significant changes were detected. No subjective or objective side effects occurred, with the exclusion of red facial flushing occasionally seen during and immediately after the infusion. In particular, no sign of water retention was observed in spite of the potent antidi-
obtained from 40 ml of citrated blood collected before the infusion of both of these properties was maximal at 30-120 min.

Samples were run at 20 mA (constant current) until the ion front reached the end of the gel (usually 5-6 hr). The temperature was maintained at 16°C throughout the run. Factor VIII procoagulant activity (VIII:C) was assayed on fresh samples by a one-stage clotting technique.22 Factor-VIII-related antigen (VIIIR:Ag) was measured by quantitative immunoelectrophoresis23 and factor-VIII-related ristocetin cofactor (VIIIR:RCo) with formalin-fixed platelets as previously described.23

The multimeric distribution of FVIII/vWF was analyzed by thin-layer agarose electrophoresis in the presence of sodium dodecyl sulfate (SDS) using a discontinuous buffer system according to a method recently described.24 All gels were 1.4% agarose. The stacking gel buffer was 0.125 M Tris-HCl, pH 6.8, and the running gel buffer 0.375 M Tris-HCl, pH 8.8. SDS was present throughout at 0.1% in both gels. Prior to running, 1 μl plasma samples were diluted in 20 μl of sample buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.0, 2% SDS, 8 μl urea) and the entire diluted sample applied. Samples were run at 20 mA (constant current) until the ion front reached the end of the gel and then at 25 mA until the tracking dye reached the end of the gel (usually 5-6 hr). The temperature was maintained at 16°C throughout the run. FVIII/vWF multimers were identified by exposing the gels to 125I-labeled affinity-purified antibodies to human FVIII/vWF followed by autoradiography.26

The bleeding time was measured by exposing the gels to 125I-labeled affinity-purified antibody. Plasma factor VIII/von Willebrand factor from a normal individual (NP) is shown on the left and compared to platelet factor VIII/von Willebrand factor from a normal (I) and from patients with von Willebrand’s disease type I, IIA, and IIB. The arrow indicates the origin of the running gel and the anode is at the bottom of the gel.

**RESULTS**

**Normal Individuals**

DDAVP produced a concomitant increase in total VIIIR:Ag and VIIIR:RCo to about two times the resting concentration (Fig. 2, bottom). The increase in both of these properties was maximal at 30-120 min. All multimers of FVIII/vWF showed an increase in plasma concentration. Larger multimers than were initially present in plasma also appeared, reaching a maximum at 1 hr (Fig. 2, top). These larger multimers were similar in size to those found to be present in platelets (Fig. 1). They disappeared from the circulation by 4 hr, even though total VIIIR:Ag had not significantly declined from peak levels and VIIIR:RCo was still appreciably increased. The increment of factor VIII procoagulant activity (VIII:C) following DDAVP was consistently greater than that of VIIIR:Ag and VIIIR:RCo in both normals and all the patients studied, as already reported.2 10

![Fig. 1. Autoradiograph pattern of factor VIII/von Willebrand factor electrophoresed in 1.4% agarose in the presence of sodium dodecyl sulfate and detected by reaction with 125I-labeled affinity-purified antibody. Plasma factor VIII/von Willebrand factor from a normal individual (NP) is shown on the left and compared to platelet factor VIII/von Willebrand factor from a normal (I) and from patients with von Willebrand’s disease type I, IIA, and IIB. The arrow indicates the origin of the running gel and the anode is at the bottom of the gel.](www.bloodjournal.org)

<table>
<thead>
<tr>
<th>Type</th>
<th>Multimeric Composition of FVIII/vWF</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Concentrations of VIII:C, VIIIR:Ag and VIIIR:RCo in plasma are decreased and to the same relative degree. Concentrations of VIIIR:Ag and VIIIR:RCo in platelets are normal, as is the multimeric composition of plasma and platelet FVIII/vWF. Ristocetin-induced FVIII/vWF-platelet interaction is qualitatively normal (the characteristic hemostatic and laboratory abnormalities result from reduced concentrations of all multimeric forms of FVIII/vWF in plasma).</td>
</tr>
<tr>
<td>II A</td>
<td>Variable concentrations of VIII:C and VIIIR:Ag in plasma (both may be normal); VIIIR:RCo is always markedly decreased or undetectable in plasma. Platelets contain normal concentrations of VIIIR:Ag, but VIIIR:RCo does not occur or is detectable and undetectable. Plasma and platelets lack large and intermediate multimeric forms of FVIII/vWF. Ristocetin-induced FVIII/vWF-platelet interaction is reduced.</td>
</tr>
<tr>
<td>IIB</td>
<td>Variable concentrations of VIII:C and VIIIR:Ag in plasma (both may be normal); VIIIR:RCo is lower than VIIIR:Ag and is usually decreased, but may be normal. Platelets contain normal concentrations of VIIIR:Ag and VIIIR:RCo. Large multimers of FVIII/vWF are absent in plasma but intermediate forms are present. In contrast, multimeric composition of platelet FVIII/vWF is normal. There is heightened ristocetin-induced FVIII/vWF-platelet interaction.</td>
</tr>
</tbody>
</table>

*An additional less common form of von Willebrand's disease is inherited from both parents and is characterized by unmeasurable or trace amounts of plasma and platelet FVIII/vWF. Treatment with DDAVP in these cases has uniformly failed to raise the plasma levels of FVIII/vWF (severe von Willebrand's disease).
Type I von Willebrand's Disease

In this form of the disease, all the FVIII/vWF multimers are present in basal conditions even though they are decreased in quantity (see Fig. 3, top). The response to DDAVP was qualitatively similar to normal, with increase in all the FVIII/vWF multimers and the appearance of larger forms than were present in the resting state in plasma. Type I patients, like normal individuals, have these larger multimers present in their platelets (Fig. 1). The changes in the plasma concentration and multimeric composition of FVIII/vWF were associated with correction of the bleeding time to normal values in three type I patients, in all of whom VIIIR:RCo and VIIIR:Ag were raised to normal levels by DDAVP infusion (Fig. 3, bottom). In an additional patient, the bleeding time was almost entirely corrected (17 min to 7:15 min; upper limit of normal 6:50 min). In one patient, correction of VIIIR:RCo and VIIIR:Ag was only partial, reaching a maximum of 30 and 34 U/dl, respectively. In this patient, bleeding time correction was also only partial (>30 min to 18 min).

Type IIA von Willebrand's Disease

In this form of von Willebrand's disease, the larger multimers are missing from plasma as in type IIA. In contrast to IIA, however, intermediate forms are present in plasma (see Figs. 5 and 6, top) and the multimeric composition of platelet FVIII/vWF is similar to that of normals (Fig. 1). Administration of DDAVP to patients with IIB von Willebrand's disease produced a dramatic alteration in plasma FVIII/vWF multimeric composition. There was a rapid (10–15 min from beginning of the infusion) appearance of larger multimers in plasma so that the multimeric composition was qualitatively no longer...
MULTIMERIC COMPOSITION OF F VIII/VWF

Fig. 3. (Top) Autoradiograph pattern of plasma factor VIII/von Willebrand factor from a patient with von Willebrand's disease type I, before (time 0) and at various times after infusion of DDAVP. The pattern of normal plasma (N) is shown for comparison on the extreme left lane. (Bottom) Quantitative changes of factor VIII/von Willebrand factor and of the bleeding time, measured with an automatic template method (Simplate), observed in the same patient before and at the same times after DDAVP.
for personal use only on November 9, 2017. For personal use only.
The multimeric composition of plasma FVIII/vWF is altered by DDAVP infusion in that multimers larger than present under basal conditions can be demonstrated. Platelet FVIII/vWF also has larger multimers than those present in plasma during the resting state. Stimulation of platelets with such agents as adenosine diphosphate (ADP) and collagen provokes release of these larger multimers. \(^*\) Though the larger multimers appearing in plasma after DDAVP infusion may have come from endothelial cells as well as (or instead of) platelets, their existence was predicted by the multimeric analysis of platelet FVIII/vWF.

In type I von Willebrand’s disease, the multimeric composition of plasma and platelet FVIII/vWF is similar to that seen in normals, even though the plasma concentration is significantly decreased. This suggests that the basic defect in this form of the disease is a reduced rate of production or release of otherwise normal FVIII/vWF. The studies reported here tend to bear out such a hypothesis. Infusion of DDAVP resulted in an increase in plasma concentrations of all multimers with a time course similar to normal. Correction of the hemostatic defect, as indicated by bleeding time normalization, was complete, or nearly so, when FVIII/vWF levels were normalized. Thus, DDAVP should be useful in preparing individuals with type I von Willebrand’s disease for surgical procedures or in treating bleeding episodes, provided that DDAVP can raise FVIII/vWF levels into the normal range.

In type IIA von Willebrand’s disease, the larger multimers are absent from both plasma and platelets, although some intermediate forms are present in the latter. DDAVP produced an almost threefold increase in plasma FVIII/vWF levels to well above the average normal concentration. Nevertheless, there was only a minimal change in the plasma FVIII/vWF multimeric composition, and though the bleeding time showed variable shortening, it was never corrected. These findings suggest that larger forms are absent not only from platelets but also from other tissue sites, and that in this subtype of von Willebrand’s disease, a defect in formation of the larger multimers is likely. Therefore, DDAVP cannot be expected to completely correct the hemostatic defect in IIA von Willebrand’s disease no matter how great the quantitative increase in plasma FVIII/vWF might be.

The FVIII/vWF in IIB von Willebrand’s disease shows an increased affinity for platelet binding sites in the presence of ristocetin.\(^{16,17}\) This has led us to postulate that the relative absence of large FVIII/vWF multimers in IIB plasma (in spite of their presence in platelets) is the result of rapid binding and removal of these large forms following release from tissue sites.\(^{16}\) The findings of this study produce direct evidence that this is the case in vivo. There is a rapid appearance of the larger multimers following infusion of DDAVP in IIB patients. However, these are rapidly cleared from the circulation—in contrast to the smaller multimers that remain elevated for some time. In type I von Willebrand’s disease, on the other hand, the large multimers that enter the circulation are cleared at a rate similar to normal. The bleeding time failed to normalize in our IIB patients, even when normal plasma levels of VIIIR:Ag and VIIIR:RCo were attained. Several different explanations may be considered. Though the larger multimers appeared in plasma, they circulated for a brief time and may never have reached sufficient concentrations to support platelet adhesion in these patients. Alternatively, the increased responsiveness of IIB FVIII/vWF to ristocetin may be indicative of a true functional abnormality in this molecule, rendering it incapable of performing

\(^*\)Lopez MF, Ruggeri ZM, Ginsberg M, and Zimmerman TS: submitted for publication.
its normal hemostatic role. In two IIB patients studied by Takahashi, bleeding time correction was seen following DDAVP, although it only lasted for 1–2 hr. Thus, it appears that as with type IIA, DDAVP will not reliably correct the hemostatic abnormality in type IIB patients, especially in mucosal bleeding where normal platelet function is particularly important.

Finally, these results suggest that platelet FVIII/vWF accurately represents the multimeric composition of FVIII/vWF present in tissue stores. Its analysis is useful in classifying the different subtypes of von Willebrand's disease, as well as in interpreting the results of therapeutic intervention.

ACKNOWLEDGMENT

We are indebted to Rossella Bader, Raffaella Coppola, Carla Velscchi, and Jim Roberts for technical assistance, and to Roberta Novak-Buhrman for typing the manuscript.

REFERENCES

1. Mannucci PM, Aberg M, Nilsson IM, Robertson B: Mechanism of plasminogen activator and factor VIII increase after vasoactive drugs. Br J Haematol 30:81, 1975
Multimeric composition of factor VIII/von Willebrand factor following administration of DDAVP: implications for pathophysiology and therapy of von Willebrand’s disease subtypes

ZM Ruggeri, PM Mannucci, R Lombardi, AB Federici and TS Zimmerman