DDAVP in Type IIa von Willebrand’s Disease

By Harvey R. Gralnick, Sybil B. Williams, Laurie P. McKeown, Margaret E. Rick, Pascal Maisonneuve, Christine Jenneau, and Yvette Sultan

1-D-Amino(8-D-arginine)-vasopressin (DDAVP) infusion in three patients with type IIa von Willebrand’s disease (vWD) resulted in a normalization of the factor VIII coagulant, factor VIII-related antigen, and von Willebrand factor (vWF) (ristocetin cofactor) activities and the bleeding time. The normalization of these hemostatic parameters persisted for four hours. Over the same time period there was a marked increase in the quantity of the vWF multimers when blood was collected in the presence of protease inhibitors. The vWF multimers present were even larger than the normal. When blood was collected in the absence of protease inhibitors, a smaller increase in the plasma vWF multimers was observed and fewer of the intermediate and larger vWF multimers were seen; multimers larger than those present in normal plasma were not visualized. The platelet vWF multimers and activities did not change with or without inhibitors. These studies suggest that there is a subgroup of patients with type IIa vWD who respond to DDAVP with complete normalization of their hemostatic abnormalities and whose vWF is sensitive to proteolysis.

Materials and Methods

Patients. Patients described in this study have been diagnosed as type IIa vWD. They have absent ristocetin-induced platelet aggregation and normal or near normal VIII R:Ag levels with marked reductions in vWF activity (ristocetin cofactor) (Table 1). They appear to be similar to the patients described as type IIa by other authors.1-3 The patients were aware of the experimental nature of the study and gave their informed consent. All experiments were performed according to the declaration of Helsinki. DDAVP (Stimate, Revlon-Armour Berkley Heights, NJ; Minirin, Ferring Pharmaceuticals, Inc, Malmo, Sweden) was administered at a dose of 0.3 μg/kg body weight; the medication was added to 50 mL of normal saline and infused intravenously over a 30-minute period. Before, during, and after the infusion period, blood pressure and pulse were checked every 30 minutes in the contralateral arm. The patients were questioned as to any subjective symptoms during or after the treatment.

Blood. Blood samples were collected by venipuncture with a 19-gauge needle, using a two-prong technique and polypropylene syringes; the blood in the first syringe was used for complete blood counts and the blood collected in the second syringe was used for coagulation studies. Two sets of tubes were used for the coagulation studies: one set of tubes contained sodium citrate 3.2% (final concentration 10.9 mmol/L) and the second set of tubes contained an identical concentration of sodium citrate with 5 mmol/L EDTA, 1 mmol/L leupeptin, and 6 mmol/L N-ethylmaleimide (NEM). The proportion of blood to anticoagulant was the same in both sets of tubes (9:1). The blood was centrifuged at 3,000 g at 4 °C for 15 minutes, and the plasma was separated and tested fresh or was frozen at −70 °C for no longer than seven days before testing. Samples were obtained before and at 30, 60, 120, and 240 minutes in two patients and at 20, 40, 60, 90, 120, 240 minutes, and 24 hours in one patient after infusion.

The VIII:C activity was assayed on fresh samples by a one-stage method based on the partial thromboplastin time as previously described.11 VIII R:Ag was assayed by electromembranodiffusion according to Laurell and the vWF activity was measured using formalinized fixed platelets as previously described.11 The multimeric organization of the vWF was analyzed by glyoxyl agarose electrophoresis in the presence of sodium dodecyl sulfate.12 The technique was modified from the original description, including a reduction in sample size to 10 μL and a change in gel thickness from 2.0 mm to 0.5 mm. Samples were run at 50 mA until the tracking dye reached 1 cm from the end of the gel. Temperature was maintained at 17 °C throughout the run. The vWF multimers were identified after incubation in the agarose gel with 125I affinity-purified rabbit antihuman vWF antibody and subsequently developed by autoradiography.

Bleeding times were performed using the technique of Ivy using a standardized template technique before the infusion of DDAVP and two hours after infusion was finished. Before and two hours after completion of the DDAVP infusion, blood was obtained for analysis of platelet vWF. Platelets were separated from plasma proteins on arabino-galactan gradients as previously described.12 The plasma-free platelets (1 × 109 per microliter) were lysed by Triton X-100 (Sigma Chemical Co, St Louis), and the supernatants of the platelet lysate were analyzed for VIII R:Ag and vWF activities and for multimeric structure by glyoxyl agarose electrophoresis (see above).

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Table 1. Type Ila vWD

<table>
<thead>
<tr>
<th>Patient</th>
<th>Bleeding Time (min)</th>
<th>VIII:C (%)</th>
<th>VIIIR:Ag (%)</th>
<th>vWF (%)</th>
<th>RIPA (mm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;20</td>
<td>38</td>
<td>67</td>
<td>20</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>35-42</td>
<td>60-72</td>
<td>12-20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&gt;20</td>
<td>38</td>
<td>34</td>
<td>7</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>29-42</td>
<td>30-48</td>
<td>5-15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&gt;30</td>
<td>31</td>
<td>44</td>
<td>7</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>26-37</td>
<td>44-60</td>
<td>5-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>&lt;8</td>
<td>48-155</td>
<td>55-168</td>
<td>59-143</td>
<td>56-76</td>
</tr>
</tbody>
</table>

Normal ranges were derived from 25 healthy subjects. Values for the patients are those obtained before the DDAVP infusion. The values underneath are the range of at least three determinations. The bleeding times have always been greater than 15 minutes. Shown here are the longest bleeding times recorded for each patient. RIPA, ristocetin-induced platelet aggregation at 1.81 mg/mL ristocetin concentration, mm/min; NA, no aggregation.

RESULTS

The three patients had similar coagulation abnormalities before the infusion of DDAVP. In each, the VIII:C was reduced, and in two patients the VIII R:Ag was reduced on the determination before the DDAVP infusion. vWF was severely decreased in all three patients, with the values ranging from 7% to 20% (Table 1). Bleeding times were prolonged (>15 minutes) in all three patients. The multimeric analyses of the plasma vWF protein were abnormal in that they lacked the largest and intermediate-sized multimers and had enhancement of the minor bands (triplet structure) of each multimer series (Fig 1). The platelet vWF multimeric structure revealed a lack of the largest multimers (data not shown).

Thirty minutes after completion of the DDAVP infusion there was a marked rise in the vWF and VIII:C activities and

*Fig 1. Agarose gel electrophoresis of plasma vWF. Plasma from normal pool (lane 1) and patients 1, 2, and 3 from Table 1 (lanes 2, 3, and 4, respectively) were analyzed by glyoxal agarose gel electrophoresis. Note that the patients lack the intermediate and larger vWF multimers compared with the normal and have increased binding of the radiolabeled antibody to minor bands of each multimer subset. Anode is at the bottom.*

Table 2. DDAVP Infusion in Patients With Type Ila vWD

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre</th>
<th>Post*</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
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</thead>
<tbody>
<tr>
<td>VIII:C (%)</td>
<td>38</td>
<td>147</td>
<td>38</td>
<td>156</td>
<td>31</td>
<td>115</td>
</tr>
<tr>
<td>VIII R:Ag (%)</td>
<td>67</td>
<td>265</td>
<td>34</td>
<td>255</td>
<td>44</td>
<td>238</td>
</tr>
<tr>
<td>vWF (%)</td>
<td>20</td>
<td>58</td>
<td>7</td>
<td>72</td>
<td>7</td>
<td>77</td>
</tr>
<tr>
<td>Bleeding time (min)</td>
<td>&gt;20</td>
<td>8.5</td>
<td>&gt;15</td>
<td>5</td>
<td>&gt;15</td>
<td>5.5</td>
</tr>
</tbody>
</table>

*Peak of VIII:C, VIII R:Ag, and vWF activity occurred 30 to 60 minutes after DDAVP infusion.*

*Fig 2. Time sequence of the changes of VIII:C, VIII R:Ag, and vWF after DDAVP infusion. (A) is Patient 1 and (B) is Patient 2 (Table 1). In both patients the vWF and VIII:C remained in the normal range for up to four hours. In one patient who was studied, the values returned to baseline at 24 hours. In both patients there was normalization of the bleeding time at two hours.*
a rise in the VIII R:Ag to normal levels. The levels of all three activities remained normal for at least four hours (Fig 2). The bleeding time, which had been prolonged (>15 minutes) before DDAVP infusion, was corrected at two hours to 5.0 minutes, 5.5 minutes, and 8.5 minutes, respectively, in each patient (Table 2 and Fig 2).

There were marked changes in the patterns of the plasma vWF multimers. This was most apparent in the blood that was collected in the presence of the protease inhibitors (Fig 3). These clearly showed that within 20 minutes after the infusion, there was a marked increase in the amount of antigen present and that larger multimers were present than previously seen in the patients' plasmas; in two out of three cases, the multimers were even larger than those seen in normal plasma. In contrast, blood collected in the absence of protease inhibitors showed only a moderate increase in the intermediate and larger-sized multimers; the largest multimers were not seen. It was also noted that the enhancement of the minor bands of the vWF multimers (seen in baseline studies in the type IIa patients) was less intense in the blood samples collected in the presence of the protease inhibitors. The multimeric structure remained normal at four hours but returned to the preinfusion pattern by 24 hours.

Analysis of the platelet vWF multimers in two of the patients two hours after infusion of DDAVP were similar to those observed before the DDAVP infusion, and the platelet VIII R:Ag and vWF activities did not change significantly two hours after the DDAVP infusion (before infusion, platelet VIII R:Ag 34% and 72%; vWF activity 15% and 6%, respectively; postinfusion, VIII R:Ag 30% and 77% and vWF activity 20% and 9%, respectively).

DISCUSSION

DDAVP has been a useful therapeutic modality for the treatment of patients with mild hemophilia or type I vWD. Previous studies using DDAVP in type IIa vWD revealed that despite elevation of the VIII R:Ag and VIII:C to the normal range, the vWF activity and the bleeding time were not corrected, and the multimeric structure of the plasma vWF was virtually unchanged. Contrary to that report, the results in our three patients indicate that at least some patients with type IIa vWD may respond to DDAVP. The increase seen in the VIII R:Ag and vWF activities is paralleled by a change in the multimeric structure of the vWF. When the blood is collected in the presence of protease inhibitors, even larger multimers than those seen in normal plasma are observed after the DDAVP infusion. These data suggest that some patients with type IIa vWD synthesize vWF in their endothelial cells that has a full complement of multimers, and that release of these multimers into the circulation occurs after DDAVP stimulation. Our data with DDAVP infusion indicate that these released multimers are also sensitive to the same enhanced proteolytic digestion as previously described in type IIa vWD.

These studies are in agreement with our previous studies reporting the full complement of multimers in patients with type IIa vWD when their blood is collected in protease inhibitors. Our data are also compatible with observations by McCarroll et al showing that DDAVP increases the vWF antigen II in type IIa vWD in a manner similar to normals and patients with type I vWD. Recent observations indicate that vWF antigen II and vWF are both synthesized in endothelial cells and form a single protein complex. The separation of the vWF antigen II from the vWF molecule results from proteolysis. It is not clear where and how the proteolysis of the vWF occurs in these patients, but it does appear to affect both the vWF antigen II and the vWF.

Thus this study suggests that some type IIa vWD patients do synthesize a full complement of multimers and do release them after DDAVP infusion. In these patients, DDAVP infusion may be a useful substitute for cryoprecipitate infusion, thus avoiding the risks associated with infusion of plasma products.

REFERENCES

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