DDAVP Shortens the Prolonged Bleeding Times of Patients With Severe von Willebrand Disease Treated With Cryoprecipitate. Evidence for a Mechanism of Action Independent of Released von Willebrand Factor

By Marco Cattaneo, Marco Moia, Patrizia Della Valle, Paola Castellana, and Pier Mannuccio Mannucci

After infusion of cryoprecipitate, the very prolonged bleeding time of patients with severe von Willebrand disease (vWD) is shortened but not always normalized in spite of normalization of plasma von Willebrand factor (vWF) levels. Therefore treatments that further improve primary hemostasis in severe vWD patients are needed. Since DDAVP shortens the bleeding time in a variety of bleeding disorders, we investigated in a double-blind, placebo-controlled crossover study the effects of the intravenous (IV) infusion of DDAVP (0.3 μg/kg) on the bleeding times of 10 patients with severe vWD treated with cryoprecipitate. Their very prolonged bleeding times (>30 minutes), partially corrected by the infusion of cryoprecipitate (14 ± 2 minutes, mean ± SEM), were further shortened by the administration of DDAVP (9 ± 2 minutes, P < .01) but not of saline (15 ± 3 minutes, ns). Plasma vWF levels, raised from unmeasurable to normal values by cryoprecipitate, were not changed after DDAVP or saline. The defective deposition of platelets from eight patients onto human umbilical artery subendothelium was increased but not normalized by cryoprecipitate and was not significantly affected by DDAVP or saline. Therefore the infusion of DDAVP after cryoprecipitate may be of clinical benefit for management of bleeding episodes in severe vWD patients. Since severe vWD patients do not have releasable tissue stores of vWF, DDAVP must shorten their prolonged bleeding times independently of released vWF.

Patients with severe von Willebrand disease (vWD) lack von Willebrand factor (vWF) in plasma and in tissues and have very prolonged bleeding times due to impaired vWF-dependent interaction of their platelets with the subendothelium of the vessel wall. The infusion of cryoprecipitate shortens but does not always normalize their prolonged bleeding times, despite normalization of plasma vWF levels. Since clinical experience indicates that mucosal bleeding in vWD patients cannot be stopped unless the bleeding time is shortened to normal values, treatments that further improve primary hemostasis in severe vWD patients would be of benefit for management of their bleeding episodes.

The synthetic analogue of vasopressin DDAVP, which causes the release of vWF from tissue stores, is a hemostatic agent because it shortens the prolonged bleeding times of patients with a variety of congenital or acquired disorders of primary hemostasis and reduces operative blood loss in patients undergoing cardiopulmonary bypass surgery or spinal fusion surgery with insertion of Harrington rods. While its effectiveness in patients with type I vWD is easily demonstrated,8,9 mechanisms both dependent and independent of released vWF have been advocated.

This double-blind, placebo-controlled crossover study was performed to see whether DDAVP shortens the bleeding time of severe vWD patients treated with cryoprecipitate. Since severe vWD patients do not have releasable vWF in tissue stores, this study provided an opportunity to see whether or not DDAVP can shorten the bleeding time independently of released vWF.

MATERIALS AND METHODS

Patients. Ten multitransfused patients with severe vWD were studied. They had unmeasurable levels of vWF antigen (vWF:Ag) and vWF:ristocetin cofactor (vWF:RCoF) in both plasma and platelets and have been previously described. They needed cryoprecipitate infusions for treatment of joint or muscle hemorrhages. All patients gave informed consent to the study and to further treatment with DDAVP or saline.

Treatment schedule. After an attempt to shorten their very prolonged bleeding times (>30 minutes) with infusion of wet cryoprecipitate (half a bag/kg) made by our hospital blood bank, severe vWD patients were randomly and blindly assigned to treatment with 0.3 μg/kg DDAVP or saline, infused intravenously (IV) over 30 minutes. DDAVP or saline infusions were started 30 minutes after the end of the infusion of cryoprecipitate to allow measurements of postcryoprecipitate bleeding times. Treatments with DDAVP or saline were crossed over after at least 15 days on occasion of new bleeding episodes needing cryoprecipitate infusion. Bleeding times were obtained, and venous blood was collected in trisodium citrate, 12.9 mmol/L (for plasma vWF measurements) and 11 mmol/L (for studies of platelet deposition onto umbilical artery subendothelium) immediately before and after infusion of cryoprecipitate and after the infusion of DDAVP or saline.

Bleeding time. Bleeding times were measured on the forearm with a template method (Symplate II, General Diagnostics, Milan, Italy) by an expert operator. Results were expressed as the average bleeding time from the two vertical incisions. Since DDAVP treatment can sometimes be detected from the mild facial flushing caused by the drug, to avoid biases in the measurement of the bleeding time, the operator was kept unaware of either the ongoing treatment or the design and purpose of the study. (She did not know that DDAVP was the drug being tested, nor did she know the expected effects of the test drug on the bleeding time.)

Measurements of plasma vWF:RCoF, and vWF multimeric analysis were carried out as described using an electroimmunoassay assay (vWF:Ag), an aggregometric assay with formalin-fixed platelet
Figure 1. Changes in bleeding times in 10 patients with severe vWD treated with infusion of cryoprecipitate (half a bag/kg), followed by the infusion of DDAVP (0.3 μg/kg, panel A) or saline (panel B). Different symbols have been used to indicate each patient. The dashed horizontal line indicates the upper limit of the normal range for the bleeding time (7 minutes).

Table 1. Effects of the Infusion of Cryoprecipitate, Followed by DDAVP or Saline on the Bleeding Times and Plasma vWF Levels of 10 Patients With Severe vWD

<table>
<thead>
<tr>
<th>Bleeding time (min)</th>
<th>Normal Range</th>
<th>After Cryoprecipitate</th>
<th>After DDAVP</th>
<th>After Cryoprecipitate</th>
<th>After Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding time (min)</td>
<td>&lt;7</td>
<td>14 ± 2</td>
<td>9 ± 2*</td>
<td>15 ± 3</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>vWF-Ag (U/dL)</td>
<td>50-150</td>
<td>75 ± 7</td>
<td>69 ± 6</td>
<td>92 ± 9</td>
<td>83 ± 10</td>
</tr>
<tr>
<td>vWF-Ricof (U/dL)</td>
<td>50-150</td>
<td>64 ± 7</td>
<td>64 ± 7</td>
<td>76 ± 8</td>
<td>63 ± 7</td>
</tr>
</tbody>
</table>

Mean is ± SEM.
Bleeding times were >30 minutes, vWF-Ag and vWF-Ricof were unmeasurable (<1 U/dL and <6.5 U/dL, respectively) before the infusion of cryoprecipitate.

*Significantly different from after cryoprecipitate (P < .01), t test for paired data.
shows that DDAVP further shortened the prolonged bleeding times of severe vWD patients after partial correction by the infusion of cryoprecipitate. Soft tissue and postsurgical bleeding times of severe vWD patients after partial correction by infusion of vWF concentrates and by the associated normalization of the factor VIII:C plasma levels independently of the normalization of the Ivy bleeding time. However, spontaneous mucosal bleedings cannot be stopped unless the bleeding can be effectively controlled in vWD patients by the infusion of cryoprecipitate. Soft tissue and postsurgical bleeding times of severe vWD patients after partial correction by infusion of vWF concentrates and by the associated normalization of the factor VIII:C plasma levels independently of the normalization of the Ivy bleeding time.

The plasma levels of vWF:Ag and vWF:RiCoF, undetectable in the baseline samples, increased to normal levels in all patients after the infusion of cryoprecipitate and did not change after the infusions of DDAVP or of saline (Table 1). The multimeric pattern of plasma vWF after the infusion of cryoprecipitate was normal (not shown); larger than normal ("supranormal") vWF multimers were not detectable after the infusion of DDAVP (not shown).

Platelet deposition onto human umbilical-artery subendothelium, expressed as coverage and as thrombus formation, was severely impaired in the eight severe vWD patients studied (Table 2). Cryoprecipitate significantly increased but did not normalize either the percentage of platelet coverage or thrombus formation (Table 2). The infusion of DDAVP or saline after cryoprecipitate did not significantly affect platelet coverage or thrombus formation (Table 2).

### DISCUSSION

This double-blind, placebo-controlled crossover study shows that DDAVP further shortened the prolonged bleeding times of severe vWD patients after partial correction by the infusion of cryoprecipitate. Soft tissue and postsurgical bleeding can be effectively controlled in vWD patients by the infusion of vWF concentrates and by the associated normalization of the factor VIII:C plasma levels independently of the normalization of the Ivy bleeding time. However, spontaneous mucosal bleedings cannot be stopped unless the Ivy bleeding time is shortened to normal values. Therefore DDAVP is of potential clinical usefulness for treatment of mucosal bleedings in patients with severe vWD who are not fully responsive to cryoprecipitate.

The results of our study also help to understand the mechanism of action of DDAVP. While the effectiveness of DDAVP in type I vWD can be explained by the associated increase of plasma vWF, the mechanism of action of the drug in subjects with normal or even increased plasma vWF levels (such as patients with uremia, liver cirrhosis, or with congenital or acquired defects of platelet function) is not easy to understand. It has been postulated that the vWF with "supranormal" multimers that is transiently released by DDAVP from tissue stores might enhance platelet adhesion onto subendothelium and thereby potentiate primary hemostasis. However, mechanisms independent of released vWF have also been advocated. Barnhart et al., for instance, showed that DDAVP in vitro enhances platelet adhesion onto the subendothelium through a mechanism not involving the release of vWF. Mechanisms independent of released vWF are difficult to explore in vivo because any subject with measurable vWF plasma levels usually releases vWF from tissue stores when infused with DDAVP. We thought that severe vWD patients with prolonged bleeding times after treatment with cryoprecipitate should be an ideal natural model to test the effects of DDAVP on bleeding time that are independent of released vWF because they have no tissue stores of vWF, since exogenous vWF does not exchange between plasma and endothelial or platelet vWF. As expected, the plasma vWF:Ag and vWF:RiCoF levels of our patients, already normal after cryoprecipitate, remained unchanged after DDAVP infusion; and "supranormal" vWF multimers, which are transiently released by DDAVP from cellular compartments in subjects with qualitatively normal vWF, did not appear. Therefore DDAVP shortens the prolonged bleeding time independently of vWF released from tissue stores.

In earlier studies we showed that DDAVP does not shorten the bleeding times of severe vWD patients, in apparent contrast with the results of the present study. However, the effect of DDAVP might have been missed in these patients not treated with cryoprecipitate because their bleeding times were not measured beyond 30 minutes and were recorded only as longer than 30 minutes. The further shortening of the bleeding time induced by DDAVP in the present study was not great, being of the same magnitude as that attained in previous studies of patients with prolonged bleeding times and normal or even increased plasma vWF levels (see Table 1 of reference 6 for review).

Being independent of released vWF, the mechanism by which DDAVP shortens the prolonged bleeding time is unknown. Although our ex vivo study showed a trend toward an increase in platelet deposition onto human umbilical artery subendothelium after infusion of severe vWD patients with DDAVP, the observed increase was not statistically significant. The difference between our results and those of Barnhart et al., who found that DDAVP increases platelet deposition onto vascular subendothelium in vitro, may be explained by the fact that the plasma drug concentrations that are attainable in vivo after the IV infusion of 0.3 ng/kg DDAVP (about 1 ng/mL) are lower than those used by Barnhart et al (7 ng/mL) in their in vitro study.

<table>
<thead>
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<th>Severe vWD Patients</th>
<th>Normal Subjects</th>
<th>Baseline</th>
<th>After Cryoprecipitate</th>
<th>After DDAVP Baseline</th>
<th>After Cryoprecipitate</th>
<th>After DDAVP Baseline</th>
<th>After Saline Baseline</th>
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<td>Coverage (%)</td>
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<td>13 ± 2</td>
<td>24 ± 3*</td>
<td>29 ± 5*</td>
<td>13 ± 2</td>
<td>27 ± 3*</td>
<td>25 ± 3*</td>
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<td>Thrombi (%)</td>
<td>6.8 ± 2</td>
<td>1 ± 0.2</td>
<td>2.5 ± 1*</td>
<td>3.3 ± 1*</td>
<td>1 ± 0.2</td>
<td>2.7 ± 1*</td>
<td>3 ± 1*</td>
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Mean is ± SEM.
*Significantly different from baseline values (P < .01), t test for paired data.

Patients

Severe vWD

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