von Willebrand Disease "Vicenza" With Larger-Than-Normal (Supranormal) von Willebrand Factor Multimers

By Pier Mannuccio Mannucci, Rossana Lombardi, Giancarlo Castaman, Judith A. Dent, Antonella Lattuada, Francesco Rodeghiero, and Theodore S. Zimmerman

When normal volunteers or patients with type I von Willebrand disease (VWD) are given desmopressin (DDAVP), a set of larger-than-normal (supranormal) von Willebrand factor (VWF) multimers, similar to those present in VWF-containing cells such as platelets megakaryocytes and endothelial cells, appear transiently in postinfusion plasma. In two kindreds with mild lifelong bleeding symptoms transmitted as an autosomal dominant trait, all ten symptomatic members (but none of the five asymptomatic members) had a supranormal multimeric structure for plasma VWF, apparently identical to that seen for postdesmopressin normal plasma. Plasma factor VIII coagulant activity (VIII:C), VWF antigen (VWF:Ag), ristocetin-induced platelet agglutination, and ristocetin cofactor (RiCof) activity were low. Platelet VWF:Ag and RiCof levels (tested for three patients only) were normal. Bleeding times were normal or slightly prolonged. The patients' platelet multimeric structure was the same as that for normal platelets. After desmopressin infusion the plasma VWF multimeric structure remained supranormal as for preinfusion plasma, with VIII:C VWF:Ag and RiCof increasing markedly over baseline values and disappearing at a normal rate. Examination of the VWF subunit composition from three of these patients indicated that proteolytic processing of their VWF did not differ from normal. This study describes the first variant of VWD with a supranormal multimeric structure.

"1988 by Grune & Stratton, Inc.

VON WILLEBRAND FACTOR (VWF), a plasma glycoprotein that has a major role in supporting the adhesion of platelets to subendothelium during hemostasis, is synthesized, stored, and secreted by endothelial cells and megakaryocytes and is also contained in platelets.1 Plasma VWF is organized into a set of multimers ranging from dimers of the 225,000-dalton subunit to very large structures containing more than 50 subunits.1 Cellular VWF has a subset of high–molecular weight multimers larger than those normally present in plasma.2,4 Larger multimers also appear transiently in plasma of normal volunteers and patients with type I von Willebrand disease (VWD) after the infusion of desmopressin (DDAVP), a synthetic derivative of the antidiuretic hormone.4 It is thought that desmopressin indirectly causes the release of larger multimers from their storage site in Weibel-Palade bodies and that proteolytic processing contributes to their subsequent disappearance from postdesmopressin plasma.4 In this paper, we demonstrate that the resting plasma of ten patients from two separate kindreds with VWD contained a subset of larger-than-normal (supranormal) VWF multimers, strikingly similar to those found in VWF-containing cells and in postdesmopressin normal plasma.

PATIENTS AND METHODS

Ten patients from two Italian kindreds, previously diagnosed as having VWD in a national multicenter survey, were reinvestigated to analyze their VWF multimeric structure. Even though both the kindreds originate from the same province (Vicenza), they have been settled for at least five generations in two areas about 80 miles apart, and there is no evidence that they are related. In both the kindreds (identified as G. and T., Fig 1) there was a positive history for mild bleeding symptoms (especially excessive blood loss after dental extractions and surgery) transmitted from parents to children with an autosomal dominant pattern of inheritance. Healthy volunteers and five asymptomatic members of the two kindreds were studied in parallel as controls.

Methods for collecting blood and preparation of platelet-poor plasma and washed platelet lysates have been published,3 with the difference that 5 mmol/L EDTA, 6 mmol/L N-ethylmaleimide, and 1 mmol/L leupeptin were added to the sodium citrate anticoagulant, to the buffers used to wash platelets free of plasma constituents, and to the washed platelet suspensions before lysis. Factor VIII coagulant activity (VIII:C), VWF antigen (VWF:Ag), and ristocetin cofactor (RiCof) activity were measured in plasma (the latter two also in platelet lysates for three patients).3 Ristocetin-induced platelet agglutination (RIPA) was evaluated in platelet-rich plasma (PRP).8 The multimeric composition of VWF was analyzed for plasma and platelets by sodium dodecyl sulfate (SDS) agarose gel electrophoresis using previously described low-resolution and high-resolution gel systems.10 Low-resolution gels better resolve large VWF multimers; high-resolution gels better resolve smaller multimers. The subunit composition of plasma VWF was analyzed (after immunoaffinity purification, reduction, and SDS-polyacrylamide gel electrophoresis) by immunoblotting with anti-VWF monoclonal antibodies and 125I-rabbit antimouse antibody.11 Relative concentrations of VWF fragments, as compared with the intact subunit, were determined by cutting out radioactive bands from the nitrocellulose blot and quantitating radioactivity in a gamma scintillation counter.11 Desmopressin (Minerin, Valeas, Milan, Italy) was infused intravenously into six patients (G.IV 3, G.V 3, G.V 5, T.II 1, T.II 2, and T.III 2).

From the A. Bianchi Bonomi Hemophilia and Thrombosis Center and Institute of Internal Medicine, University of Milano, Italy; the Hemophilia Center and the Hematology Division, Hospital of Vicenza, Italy; and the Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, CA.

Submitted May 20, 1987; accepted August 24, 1987.

Supported in part by Consiglio Nazionale delle Ricerche (P.M.M.) Progetto Finalizzato Ingegneria Genetica, Sottoprogetto Basi Molecolari delle Malattie Ereditarie; and by Grants No. HL 31950 and HL 15491 (T.S.Z.) from the National Institutes of Health.


Address reprint requests to Pier Mannuccio Mannucci, MD, Via Pace 9, 20122 Milano, Italy.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1988 by Grune & Stratton, Inc.

0006-4971/88/7101-0008$3.00/0


65
TABLE 1. Bleeding Times, Plasma Factor VIII and von Willebrand Levels, and Multimeric Structure in Kindreds G. and T.

<table>
<thead>
<tr>
<th>Kindred, Generation, and Patient</th>
<th>Bleeding Time (min)</th>
<th>RIPA (mg/mL)</th>
<th>Platelet Count</th>
<th>Plasma VWF:Ag (U/dL)</th>
<th>RCoF (U/dL)</th>
<th>VIII:C (U/dL)</th>
<th>Multimeric Structure</th>
<th>Bleeding Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.IV 1</td>
<td>9</td>
<td>&gt;2.0</td>
<td>267,000</td>
<td>9</td>
<td>7</td>
<td>14</td>
<td>Supranormal</td>
<td>PO bleeding</td>
</tr>
<tr>
<td>G.IV 2†</td>
<td>4</td>
<td>0.7</td>
<td>236,000</td>
<td>84</td>
<td>85</td>
<td>120</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>G.IV 3</td>
<td>6</td>
<td>&gt;2.0</td>
<td>216,000</td>
<td>16</td>
<td>10</td>
<td>31</td>
<td>Supranormal</td>
<td>PO bleeding</td>
</tr>
<tr>
<td>G.IV 4</td>
<td>12</td>
<td>1.5</td>
<td>174,000</td>
<td>12</td>
<td>9</td>
<td>12</td>
<td>Supranormal</td>
<td>PO bleeding</td>
</tr>
<tr>
<td>G.IV 5</td>
<td>5</td>
<td>1.8</td>
<td>258,000</td>
<td>8</td>
<td>8</td>
<td>12</td>
<td>Supranormal</td>
<td>PO bleeding and epistaxis</td>
</tr>
<tr>
<td>G.V 1</td>
<td>5</td>
<td>1.2</td>
<td>202,000</td>
<td>81</td>
<td>70</td>
<td>80</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>G.V 2</td>
<td>6</td>
<td>1.2</td>
<td>199,000</td>
<td>58</td>
<td>60</td>
<td>78</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>G.V 3</td>
<td>5</td>
<td>1.3</td>
<td>244,000</td>
<td>10</td>
<td>10</td>
<td>14</td>
<td>Supranormal</td>
<td>Epistaxis</td>
</tr>
<tr>
<td>G.V 4</td>
<td>7</td>
<td>0.9</td>
<td>359,000</td>
<td>150</td>
<td>82</td>
<td>128</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>G.V 6</td>
<td>8</td>
<td>1.8</td>
<td>213,000</td>
<td>6</td>
<td>6</td>
<td>11</td>
<td>Supranormal</td>
<td>PO bleeding</td>
</tr>
<tr>
<td>T.II 1</td>
<td>14</td>
<td>&gt;2.0</td>
<td>300,000</td>
<td>10</td>
<td>7</td>
<td>9</td>
<td>Supranormal</td>
<td>Menorrhagia</td>
</tr>
<tr>
<td>T.II 2</td>
<td>11</td>
<td>1.5</td>
<td>208,000</td>
<td>12</td>
<td>13</td>
<td>22</td>
<td>Supranormal</td>
<td>Postpartum bleeding</td>
</tr>
<tr>
<td>T.III 1</td>
<td>ND</td>
<td>ND</td>
<td>268,000</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>Supranormal</td>
<td>PO bleeding</td>
</tr>
<tr>
<td>T.III 2</td>
<td>ND</td>
<td>ND</td>
<td>250,000</td>
<td>98</td>
<td>60</td>
<td>70</td>
<td>Normal</td>
<td>None</td>
</tr>
</tbody>
</table>

Normal range: 3-7, 0.7-1.2, 150,000-400,000, 52-160, 53-135, 53-162

Abbreviations: ND, assay not done; PO, postoperative.

*Supranormal multimeric structure denotes the presence of larger-than-normal high-molecular weight multimers.
†Asymptomatic members of the two families are in italics.
Effects of desmopressin on plasma VWF levels and multimeric structure. For the six patients studied, VWF:Ag and RiCoF (and VIII:C) increased markedly over baseline values and reached normal values 30 to 60 minutes after the infusion started. After desmopressin the mean peak levels of VIII/VWF measurements were not significantly different from those for seven congenital type I vWD patients taken as a control group (Table 2). Similarly, the mean half-disappearance times of VIII/VWF were not significantly different in the two groups (Table 2). The
Fig 4. Autoradiograph pattern of plasma (pla) and platelet (pIt) VWF in 0.8% low-gelling temperature agarose. From left to right: normal plasma, normal plasma after desmopressin, G.IV 3 plasma, platelets from G.IV 1 and T.II 1, and normal platelets. For more details, see Fig 2.

Fig 5. Autoradiograph pattern of plasma VWF for a normal subject, G.V 3, and T.II 1 electrophoresed in 2.2% low-gelling temperature agarose (high-resolution gel resolving each smaller multimer into five bands, indicated by the parenthesis). For more details, see Fig 2.

bleeding time shortened from 14 to nine minutes and from ten to six minutes in the only two patients (T.II 1 and T.II 2) in whom it was prolonged before desmopressin administration. The multimeric structure remained identical to that for patients' resting plasma and to that for postdesmopressin normal plasma (Fig 6). There was no postdesmopressin change in platelet count (not shown).

Subunit composition of plasma VWF. Examination of the subunit composition of plasma VWF from three of the affected family members demonstrated the presence of the same 189-, 176-, and 140-kd fragments as are present in normal plasma. No new fragments were demonstrated, nor was there any evidence of uncleaved pro-VWF (not shown). The intact 225-kd subunit composed between 68.2% and 69.8% of the total VWF in the patients, whereas it constituted between 70.3% and 84.5% in 25 normal individuals. The 189-kd fragment was between 7.2% and 9.4% (normal, 2.1% to 5.5%). The 176-kd fragment was between 20.6% and 21.4% (normal, 8.2% to 19.7%). The 140-kd fragment was between 1.5% and 1.8% (normal, 2.0% to 7.4%). Hence, the relative concentration of the 189- and 176-kd fragments was marginally greater than that in normal individuals, and the intact 225-kd subunit and the 140-kd fragment were marginally less.

DISCUSSION

The symptomatic members of the two kindreds had congenital VWD, as indicated by low plasma VWF levels in all

Table 2. Quantitative Changes and Half-Disappearance Times (t 1/2) of VIII/VWF (Measured as VIII:C, VWF:Ag, and RiCof) After Desmopressin Infusion in Patients With VWD Vicenza Compared With Patients With Type I VWD (platelet normal)

<table>
<thead>
<tr>
<th></th>
<th>VIII:C</th>
<th>VWF:Ag</th>
<th>RiCof</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak Level</td>
<td>t 1/2</td>
<td>Peak Level</td>
</tr>
<tr>
<td>VWD Vicenza (n = 6)</td>
<td>10.2 ± 4.9</td>
<td>76 ± 8</td>
<td>8.3 ± 3.0</td>
</tr>
<tr>
<td>Type I VWD (n = 7)</td>
<td>10.3 ± 2.3</td>
<td>68 ± 13</td>
<td>9.0 ± 2.3</td>
</tr>
</tbody>
</table>

*Expressed as ratios of peak postdesmopressin levels to baseline levels (mean ± SD).
†Expressed in minutes (mean ± SD).
cells in shear stress induced platelet aggregation. J Clin Invest before and after desmopressin infusion. For more details, see Fig 46:185, 1986


REFERENCES


Fig 6. Autoradiograph pattern of plasma VWF for G.V 3 before and after desmopressin infusion. For more details, see Fig 2.

von Willebrand disease "Vicenza" with larger-than-normal (supranormal) von Willebrand factor multimers

PM Mannucci, R Lombardi, G Castaman, JA Dent, A Lattuada, F Rodeghiero and TS Zimmerman