The Effect of DDAVP on Plasma Levels of von Willebrand Antigen II in Normal Individuals and Patients With von Willebrand's Disease

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The infusion of 1-deamino-(8-D-arginine)-vasopressin (DDAVP) causes not only an elevation in factor VIII-related antigen (FVIIIR:Ag), but also a marked elevation of plasma von Willebrand antigen II (vWAgII). vWAgII reaches a peak concentration at 60 min and is elevated 3-8-fold over basal levels in normal individuals and individuals with type I, IIA, and IIB von Willebrand's disease. As the mechanism of hemostatic alteration brought about by DDAVP might be due to release of endothelial cell proteins, endothelial cell cultures were performed. The cultures demonstrated synthesis and secretion of vWAgII, as evidenced by the incorporation of $^{35}$S-methionine into the vWAgII molecule. Thus, vWAgII, like FVIIIR:Ag, is an endothelial cell protein.

VON WILLEBRAND ANTIGEN II (vWAgII) is a protein that is immunochemically distinct from factor VIII-related antigen (FVIIIR:Ag) and is undetectable in the plasma and platelets of patients with severe homozygous (type III) von Willebrand's disease (vWD). Recent descriptions have subclassified moderate forms of vWD into types I, IIA, IIB, and IIC based on the multimeric structure of FVIIIR:Ag. The levels of vWAgII and FVIIIR:Ag were found to be correlated in the plasma of normal individuals and in patients with type I vWD, but not in patients with IIA, IIB, or in one patient with type IIC vWD.

Previous studies also demonstrated vWAgII release from platelets during aggregation with thrombin and collagen, thereby suggesting vWAgII was an α granule protein like FVIIIR:Ag. In patients with disseminated intravascular coagulation (DIC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP), the vWAgII levels were increased. However, the plasma levels attained were in excess of those that could have resulted from platelet release alone. This suggests the release of vWAgII from sites other than platelets and prompted the study of vascular endothelium. Although lysates of washed umbilical vein endothelium contained vWAgII, it was not known whether the vWAgII was adsorbed to the cells, absorbed into the cells, or represented a platelet and/or plasma contaminant.

Studies with 1-deamino-(8-D-arginine)-vasopressin (DDAVP) have demonstrated the release into plasma of proteins such as plasminogen activator and FVIIIR:Ag. Although these proteins are synthesized in endothelial cells, the site(s) of release has not been established. In types I, IIA, and IIB vWD, a quantitative increase in plasma FVIIIR:Ag levels occurred after DDAVP administration. The qualitative abnormality of FVIIIR:Ag was transient and partially corrected in IIB vWD, but was not corrected in IIA.

The present study was undertaken to determine whether DDAVP caused an increase in the plasma level of vWAgII and whether vWAgII, like FVIIIR:Ag, was not only present, but was also synthesized in the endothelial cell.

MATERIALS AND METHODS

Patient Studies

This study evaluated two normal individuals and three patients from Milan, Italy, with previously diagnosed von Willebrand's disease. These patients were classified as type I, IIA, or IIB, using the criteria previously described. Each individual received an infusion of 0.4 μg/kg of DDAVP (Minirin, Valeas, Milan, Italy) in 100 ml of normal saline over a 30-min period. The response of plasma FVIIIR:Ag in one of these patients (type IIA) has been reported elsewhere (ref 14, Fig. 4). The patient with type IIB vWD is the son of the patient described in Fig. 6 of reference 14.

Samples of citrated whole blood were obtained before and after the DDAVP infusion. Aliquots of platelet-poor plasma were frozen and stored at −80°C until they were shipped to Milwaukee for vWAgII assay.

Antisera to vWAgII

The vWAgII was obtained from the supernate of thrombin-released washed normal platelets. Outdated human platelet concentrates in ACD were washed 3 times with Tris-buffered saline (0.1 M...
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Tris-HCl, 0.15 M NaCl, pH 7.4) and then activated with thrombin (2 U/ml) for 5 min at 37°C. Following centrifugation, the soluble releasate was concentrated 8–10-fold with an Amicon B-15 Minicon concentrator (Amicon Corporation, Lexington, KY). This material was then gel-filtered through Sephacyrl S300 (Pharmacia, Piscataway, NJ) in a 1.6 x 90 cm column and the vWAgII fractions collected. New Zealand white rabbits were injected with these column fractions after 20-fold concentration in an Amicon concentrator. The antisera were absorbed with plasma from a patient with severe vWD. The IgG fraction was obtained by ammonium sulfate precipitation and DEAE ion-exchange chromatography, as previously described. The antibody was then immune-adsorbed to purified vWAgII coupled to cyanogen-bromide-activated Sepharose CL4B. Following its elution with a pH gradient, the antibody to vWAgII was radiolabeled with 121I by the chloramine-T method.

Quantitation of vWAgII and FVIIIR:Ag

The quantitation of vWAgII was performed as previously described, except that the agarose contained a mixture of cold antibody to vWAgII and radiolabeled antibody (1–2 x 10^6 cpm/plate). Plates were washed in 0.15 M NaCl with 1% Triton X100 (Sigma Chemical Company, St. Louis, MO), dried, and autoradiographed overnight. Quantitation of FVIIIR:Ag was performed as previously described. The normal plasma consisted of pooled normal plasma. One unit of vWAgII or FVIIIR:Ag is the amount of each antigen that is present in 1 ml of this pooled normal plasma. The normal range for plasma vWAgII was 40–170 U/dl. Values expressed were the average of duplicate determinations. In some experiments, vWAgII labeled with 35S-methionine and 35S-cysteine was quantitated. When radiolabeled antigen was studied, the radiolabeled antibody was omitted.

Synthesis of vWAgII by Cultured Human Endothelial Cells

Cell lysates and spent media from normal human umbilical vein endothelial cell cultures were provided by Eugene L. Levin, Ph.D. (Department of Basic and Clinical Research, Research Institute of Scripps Clinic, La Jolla, CA) and were also obtained from cultures subsequently grown in Milwaukee. Confluent 75 cm² primary cultures containing 8 x 10⁶ cells were labeled with 200 μCi/ml 35S-methionine and 100 μCi/ml 35S-cysteine (labeled cysteine was not used with Milwaukee cultures) for 4 hr in 2 ml of serum-free RPMI 1640 media (Grand Island Biologicals Company, Grand Island, NY). Media was supplemented with complete amino acids (Selectamine; GIBCO) excluding the respective radiolabeled amino acids. Samples of supernatant media were removed and frozen for assay. Cell lysates were obtained by the subsequent lysis of washed, labeled endothelial cells with 0.4 ml of 2.5% Nonidet P 40 (Particle Data Laboratories, Ltd., Elmhurst, IL). Quantitative immunoelectrophoresis and crossed-immunoelectrophoresis were carried out as previously described, and autoradiography performed as described above.

RESULTS

Quantitation of vWAgII

Use of radioimmunoelectrophoresis allowed a significant increase in sensitivity for determination of vWAgII over previous methods. The quantitation of vWAgII using radiolabeled antibody enables the detection of vWAgII at levels >0.01 U/dl. Results were linear between 3 and 100 U/dl. Figure 1 demonstrates the response of plasma vWAgII in one of the normal individuals following the infusion of DDAVP. Quantitation was performed using dilutions of patient samples.

Effect of DDAVP on Plasma vWAgII In Vivo

Each of the study individuals responded similarly to the infusion of DDAVP, as shown in Fig. 2. Both vWAgII and FVIIIR:Ag were significantly increased and reached a maximum approximately 60 min after the DDAVP infusion. The peak response of vWAgII in these individuals was 330–500 U/dl, whereas the FVIIIR:Ag was 100–220 U/dl. Following the infusion of DDAVP, the levels attained were correlated with a ratio of vWAgII to FVIIIR:Ag of 2.5 ± 0.7. Table 1 compares the pre- and postinfusion levels of vWAgII and FVIIIR:Ag. The mobility of vWAgII on crossed-immunoelectrophoresis was unaffected, regardless of the type of von Willebrand’s disease.

Synthesis of vWAgII by Cultured Human Endothelial Cells

Following a 4-hr labeling with 35S-methionine, endothelial cell lysates and culture media contained radiolabeled vWAgII. This was demonstrated using quantitative immunoelectrophoresis and crossed-immuno-electrophoresis. Figure 3 presents the results obtained when labeled endothelial cell culture supernatant is subjected to crossed-immuno-electrophoresis. Plates were autoradiographed for 2 days and demonstrate 35S-methionine incorporation into endothelial cell vWAgII. Although precise quantitation of the specific
Fig. 2. Responses of vWAgII and FVIIIIR:Ag to infusion of DDAVP were quantitated by immunoelectrophoresis. Samples were from individuals who were either normal (---- and ...) or had type I (--.---- and ... --)), type IIA (--), or type IIB (----------) vWD. The amount of each antigen was expressed in units per deciliter.

Fig. 3. Crossed-immunoelectrophoresis of 100 μl of endothelial cell culture supernatant was studied using unlabeled antibody to vWAgII in the second dimension. The culture supernatant was from primary human umbilical vein endothelial cell cultures labeled for 4 hr with 35S-methionine. This is an autoradiograph demonstrating the incorporation of 35S-methionine into the vWAgII molecule.

**DISCUSSION**

A number of endothelial cell constituents are released in vivo by DDAVP; these include plasminogen activator, \( \text{FVIIIR:Ag} \), and possibly PGI₂. It has not been demonstrated, however, that the endothelial cell is the source for these increases in plasma constituents. Of particular interest is the therapeutic effect of DDAVP in patients with von Willebrand’s disease, mild hemophilia A, and more recently, uremia. We previously demonstrated that vWAgII was deficient in type I vWD and, like FVIIIR:Ag, was an \( \alpha \) granule protein in platelets. Now, we also demonstrate that vWAgII is released by DDAVP in vivo and is synthesized in culture by endothelial cells. vWAgII lacks any demonstrable immunologic relationship with FVIIIR:Ag in plasma, serum, platelets, or endothelial cell culture supernatant. Although any functional relationship of vWAgII to FVIIIR:Ag is still speculative, caution must be exercised in concluding that the clinical effect of DDAVP in vWD is due solely to the released FVIIIR:Ag.

The demonstration of vWAgII synthesis by cultured endothelial cells suggests that the DDAVP effect on plasma vWAgII may be augmented by the release of vWAgII from the vascular endothelium. Not only do endothelial cells in culture release synthesized vWAgII, but normal endothelial cells release significant amounts into the culture medium: 1.5 U/10⁷ cells in 4 hr. Platelets, on the other hand, contain approximately 3 U vWAgII/10⁹ platelets.

As the amount of vWAgII released in vivo in response to DDAVP was 3-8 times baseline concentration, this, together with the potential platelet-released vWAgII, does not account for the massive amounts found in the plasma of some patients with DIC (>100 times normal). Thus, the levels seen in DIC might be the result of direct endothelial cellular damage. As the plasma half-life of vWAgII is not known, diminished

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**Table 1. Plasma Response of vWAgII and FVIIIIR:Ag to DDAVP**

<table>
<thead>
<tr>
<th>Individual</th>
<th>Type</th>
<th>vWAgII Ratio</th>
<th>FVIIIIR:Ag Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>3.5</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>Type I vWD</td>
<td>8.3</td>
<td>7.1</td>
</tr>
<tr>
<td>4</td>
<td>Type IIA vWD</td>
<td>5.8</td>
<td>5.7</td>
</tr>
<tr>
<td>5</td>
<td>Type IIB vWD</td>
<td>2.9</td>
<td>4.4</td>
</tr>
</tbody>
</table>

*DDAVP response ratio = maximal concentration after DDAVP/baseline concentration.
†Time following DDAVP.
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clearance rates by the reticuloendothelial system might also account for this phenomenon.

With the exception of type IIA or type IIB vWD, the correlation between the plasma levels of vWAgII and FVIIIR:Ag suggests a relationship between these molecules. With the initial description of vWAgII, we speculated that vWAgII and FVIIIR:Ag might be products of an unidentified common precursor. No such extracellular precursor has been demonstrated. Important recent studies have been carried out and have identified the monomeric precursor of FVIIIR:Ag and the intracellular biosynthesis of polymeric FVIIIR:Ag. Thus, the intracellular relationship between vWAgII and the monomeric FVIIIR:Ag molecule must be studied to determine whether a possible common precursor for these two molecules exists. An explanation for the codeficiency of these molecules in von Willebrand’s disease must still be found.

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REFERENCES

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