American Journal of Hematology 43:5–9 (1993)

Desmopressin-Induced Thrombocytopenia in Type I Platelet Discordant von Willebrand Disease

Giancarlo Castaman, Francesco Rodeghiero, Antonella Lattuada, and Pier Mannuccio Mannucci

Department of Hematology and Hemophilia and Thrombosis Center, San Bortolo Hospital, Vicenza (G.C., F.R.); Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Institute of Internal Medicine, IRCCS Maggiore Hospital and University, Milan (A.L., P.M.M.), Italy

Thrombocytopenia after desmopressin (DDAVP) infusion is usually observed in patients with type IIB von Willebrand disease (vWD). No other subtypes of vWD with thrombocytopenia after DDAVP have been reported so far. We describe here the occurrence of thrombocytopenia after DDAVP in a 39 year old male and his son with phenotypic characteristics of type I vWD, "platelet discordant subtype." After DDAVP, the abnormal ristocetin cofactor/von Willebrand factor antigen ratio in plasma was not corrected and the bleeding time remained markedly prolonged. Platelet count dropped 30 min after DDAVP (from 279 to 96 $\times$ $10^3/\mu L$ in the propositus and from 298 to 116 $\times$ $10^3/\mu L$ in his affected son) and returned to normal at 60 min. Platelet clumping was evident on peripheral blood smears obtained after infusion. These cases indicate that after DDAVP thrombocytopenia can occur in vWD other than type IIB. © 1993 Wiley-Liss, Inc.

Key words: DDAVP, post-DDAVP thrombocytopenia, ristocetin cofactor

INTRODUCTION

The vasopressin analogue desmopressin (DDAVP), which induces a substantial increase of factor VIII/von Willebrand factor and normalizes the prolonged bleeding time (BT), is the treatment of choice in the majority of patients with von Willebrand disease (vWD) [1]. DDAVP avoids the use of blood derivatives, with the inherent risk of transmission of blood-borne viruses [2]. However, DDAVP is generally contraindicated in patients with type IIB vWD, since it induces transiently a moderate to severe thrombocytopenia due to the release from stores of an abnormal von Willebrand factor (vWF) with high affinity for platelet membrane receptors [3]. No other subtypes of vWD with post-DDAVP thrombocytopenia have been reported so far. Type I vWD subtype "platelet discordant" is characterized by the disproportionate reduction of ristocetin cofactor activity (RiCof) in comparison to the level of vWF antigen, both in platelets and in plasma [4]. This subtype is due to the presence of an abnormal vWF characterized by a relative reduction of the proportion of high molecular weight multimers both in plasma and in platelets, with an apparently normal inner structure of plasma or platelet smaller multimers [4]. After DDAVP infusion, RiCof in plasma remains lower than vWF antigen and the BT remains prolonged [4]. In this paper we report the occurrence of thrombocytopenia after DDAVP infusion in a family with type I vWD platelet discordant.

MATERIALS AND METHODS

Case Report

The propositus was 39 years old when he was first referred to the Hemophilia and Thrombosis Center of Vicenza for evaluation of a moderate lifelong bleeding history. Since his childhood, he had suffered from profuse epistaxis and easy bruising. Tooth extraction was followed by prolonged bleeding. Two of his children (II.1 and II.3) suffered from epistaxis and easy bruising. His wife and a third child (II.2) were asymptomatic.

Received for publication May 20, 1992; accepted August 13, 1992.

Address reprint requests to Dr. Giancarlo Castaman, Department of Hematology and Hemophilia and Thrombosis Center, San Bortolo Hospital, 36100 Vicenza, Italy.

TABLE I. Laboratory Data for the Propositus and His Family Members*

Family member	Bleeding history	Bleeding time (min)	VIII:C (IU/dL)	vWF:Ag (IU/ dL)	RiCof (IU/dL)	RIPA (mg/mL)
Propositus	Epistaxis, easy bruising, bleeding after tooth extraction	14 to >20	43–56	25–36	<3–10	1.0–1.35
II.1	Epistaxis, easy bruising	18 to >20	38–60	19–40	<3–18	1.1–1.30
II.2	None	6.5	125	145	153	0.9
II.3	Epistaxis, easy bruising	ND	22–44	11–34	<3–15	1.1–1.35
Normal		<8	52–167	49–169	54–157	0.78–1.15

*Data represent the range of 5 separate determinations for propositus and family.

Methods

Blood was collected into 3.8% sodium citrate and centrifuged at 2,500*g* for 15 min for plasma analysis. In experiments carried out to assess in vivo proteolysis of vWF, a mixture of 5 mM EDTA, 6 mM N-ethylmaleimide, and 1 mM leupeptin (Sigma, St. Louis, Missouri) was added to citrate to avoid proteolytic degradation of vWF in vitro.

The BT was measured with the Simplate II device (General Diagnostics, Morris Plains, New Jersey), making two vertical incisions on the volar surface of the forearm. Factor VIII coagulant activity (F VIII:C) was assayed by a one-stage method and von Willebrand factor antigen (vWF:Ag) by ELISA, using a polyclonal antiserum (Stago, Paris) [5]. Ristocetin cofactor activity of vWF was measured using formalin-fixed platelets, as previously described [6]. All VIII/vWF measurements were expressed as international units (IU) after calibration of a normal pool against the 2nd International Reference Preparation for factor VIII/von Willebrand factor-related activities (provided by Institute for Biological Standards, UK). Ristocetin (RIPA) and botrocetin-induced platelet aggregation were evaluated by adding increasing amounts of ristocetin or purified botrocetin (kindly provided by Dr. Z. Ruggeri, La Jolla, California) to platelet-rich plasma and measuring the extent of aggregation 3 min later.

The multimeric composition of vWF was analysed by sodium dodecyl sulphate (SDS)–agarose gel electrophoresis in low (0.8% or 0.9% low-gelling temperature agarose) and high-resolution (2% low-gelling temperature agarose) gel systems, as previously described [7]. In normal plasma, low-resolution systems resolve each smaller multimer as a single band whereas high-resolution systems resolve each multimer into five bands, two migrating above and two below a most intense central band.

The subunit composition of plasma vWF was analysed (following immunoaffinity purification, reduction, and SDS-polyacrylamide gel electrophoresis) by immunoblotting with a panel of anti-human vWF monoclonal antibodies and ^{125}I-rabbit anti-mouse antibodies. Relative concentrations of vWF fragments were compared with the intact subunit by quantitating the radioactivity of the bands excised from the nitrocellulose blot in a gamma scintillation counter.

Platelet vWF was determined after platelet isolation on Ficoll-Hypaque gradients. Lysis was obtained by the addition of 1/40 volume of 20% Triton X-100 [8]. The supernatant was snap-frozen at −80°C. vWF measurements and multimeric analysis were performed within 2 weeks [9].

Spontaneous platelet aggregation of propositus platelet-rich plasma (PRP) was assessed by stirring PRP in an aggregometer and recording the aggregation curve for 15 min. Binding of plasma vWF to washed platelets was measured in the presence of various amounts of ristocetin or botrocetin. Platelets were washed according to the albumin density-gradient method, as previously described [10]. Residual concentration of vWF:Ag in supernatants was expressed as percentage of a control mixture with ristocetin or botrocetin replaced by buffer.

DDAVP Infusion

DDAVP was first administered as a test infusion in the propositus to assess its usefulness to cover a dental extraction. After we found thrombocytopenia, the propositus was explained the investigational purpose of the repetition of DDAVP administration and he gave informed consent for himself and his 14 year old child (II.1).

DDAVP (Minirin, Valeas, Milan) was infused intravenously over 30 min at a dose of 0.3 μg/kg b.w. · BT; VIII:C and vWF were measured before and at various times after infusion. Platelet counts were performed in EDTA- and heparin-anticoagulated samples. Peripheral blood smears were also obtained.

RESULTS

The clinical and laboratory data for the propositus and his relatives, consistent with a diagnosis of type I vWD, are summarized in Table 1 Ristocetin-induced platelet aggregation was slightly reduced in the propositus and his children. Botrocetin-induced platelet aggregation was

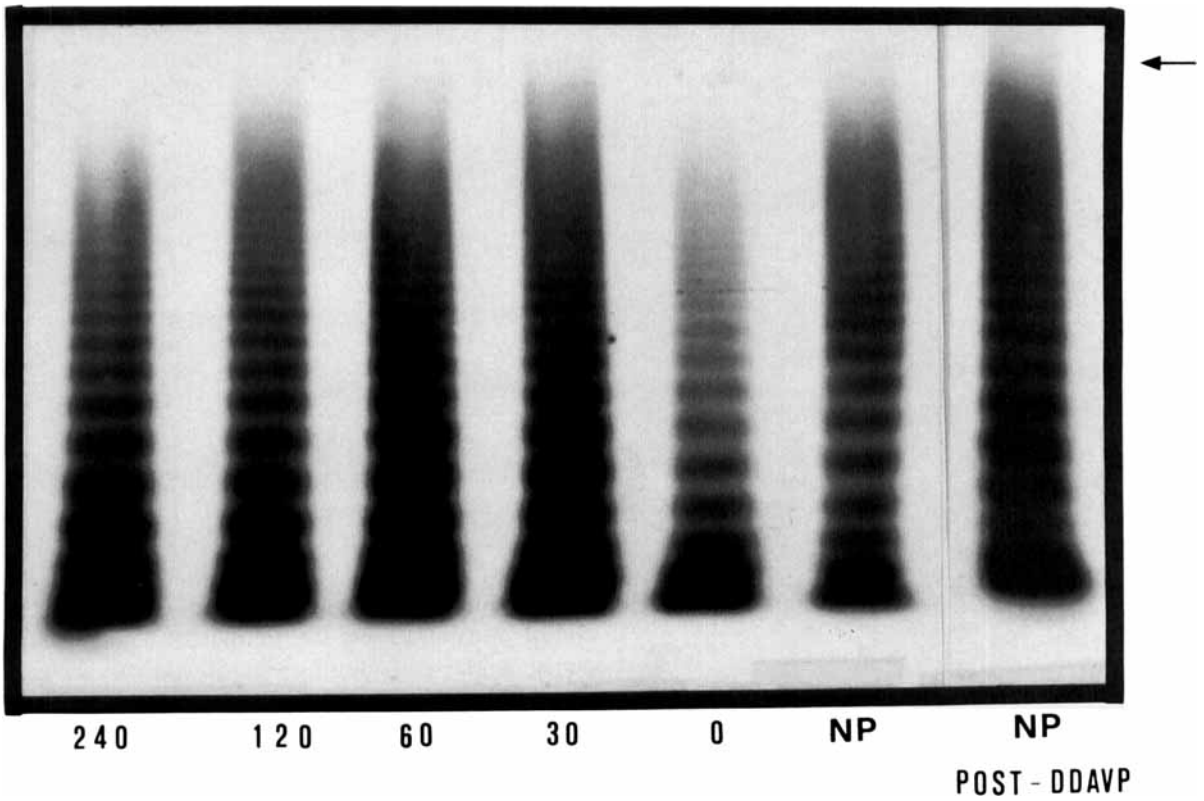

Fig. 1. SDS gel electrophoresis in a low-resolution gel (0.9% low-gelling temperature agarose). Propositus plasma (0) shows the reduction of largest vWF multimers, present in normal plasma (NP). After DDAVP, larger forms appeared in plasma, but less than in normal plasma after DDAVP (NP POST-DDAVP), and at 60 min they were already diminished. The arrow at the top indicates the interface between stacking and running gel.

low in the propositus, with 30% of aggregation occurring at 1.3 μg/mL of botrocetin (normal range 0.8–1 μg/mL).

Platelet vWF:Ag content of the propositus and his son II.1 was normal (0.26 and 0.31 IU/10^9 platelets; normal range 0.21–0.59) whereas RiCof was markedly reduced (0.04 and 0.06 IU/10^9 platelets; normal range 0.27–0.81).

Multimeric analysis on low-resolution gel of plasma vWF of the propositus and his affected children (not shown) revealed the presence of all multimers, but there was a relative reduction of the proportion of larger multimers (Fig. 1). This pattern was also present in platelet lysates (not shown). A normal pattern of plasma and platelet vWF was observed in the propositus wife and his son II.2 (data not shown). High-resolution gel electrophoresis revealed the presence of all five bands of each smaller multimer as in normal plasma, with no abnormality of subbands (not shown).

Plasma vWF subunit analysis did not reveal increased or reduced proteolysis or additional fragments. The intact 225 kd subunit was 80% and 80.5% of total vWF in the propositus and his son II.1 (74–86% in 25 normal individuals). The 189 kd fragment was 3.7% and 2.2% (normal range 1.9–5.8%). The 176 kd fragment was 11.6% and 13.4% (normal range 5.5–12.6%) and the 140 kd 4.5% and 3.8% (normal range 4–8%).

After DDAVP infusion, larger multimers were released into circulation, restoring a pattern similar to that seen in normal basal plasma, but the largest multimers, usually elicited by DDAVP in normal individuals, were not completely evident postinfusion (Fig. 1). All VIII/vWF measurements increased markedly over their basal values, but the RiCof/vWF:Ag ratio remained low and the BT prolonged (Table II). The platelet count showed a reduction at the end of infusion, in samples collected both in heparin and in EDTA (Table II), with a subsequent rapid normalization. Peripheral blood smears showed the presence of platelet clumping at 30 and 60 min after starting DDAVP and separate platelets at the end of the test. This pattern occurred on the occasion of two different DDAVP infusions. Also his son II.1 showed a similar pattern of changes of multimeric pattern (data not shown) and of vWF measurements after DDAVP (Table II).

To exclude a variant type IIB vWD, the ristocetin-induced binding of propositus plasma vWF to normal platelets was compared with that from a patient with classical type IIB vWD. Whereas the type IIB vWF gave clearly increased platelet binding, the propositus vWF

TABLE II. Laboratory Findings Before and After DDAVP Infusion

Time (min)	Bleeding time (min)	VIII:C (IU/dL)	vWF:Ag (IU/dL)	RiCof (IU/dL)	RiCof/Ag ratio	Platelets ($\times 10^3/\mu L$)
Propositus						
0	>20	42	29	<6	0.21	279
30	17	107	86	20	0.23	96
60	—	149	146	38	0.26	162
120	—	121	125	33	0.26	184
240	—	107	103	29	0.28	209
Son II.1						
0	19	58	38	12	0.31	298
30	19	134	111	34	0.31	116
60	—	171	147	41	0.28	150
120	—	93	131	39	0.30	197

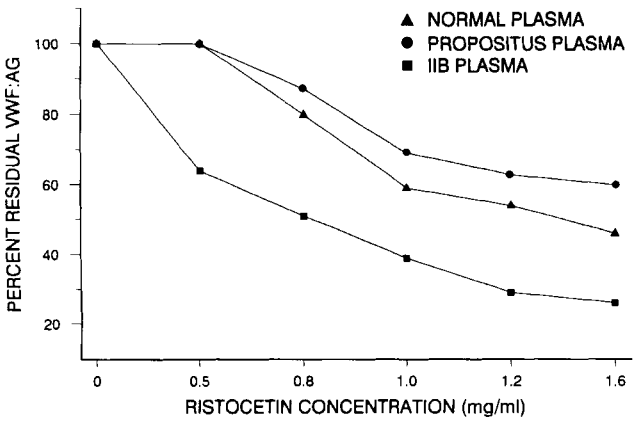

Fig. 2. Ristocetin-induced binding to normal washed platelets of plasma vWF:Ag from propositus, normal control, and a patient with type IIB vWD.

had a normal pattern (Fig. 2). Propositus and normal washed platelets incubated with normal vWF at various ristocetin concentrations showed comparable binding affinity. Spontaneous platelet aggregation was absent and the basal and post-DDAVP propositus plasma did not elicit platelet aggregation of normal or propositus platelets (data not shown).

DISCUSSION

On the basis of vWF platelet content, type I vWD can be further classified into platelet normal, platelet low, and platelet discordant [4]. Platelet discordant appears to be a rare subtype, transmitted in an autosomal dominant fashion, characterized by the presence of an abnormal vWF molecule expressed phenotypically by a normal platelet content of vWF:Ag and a marked reduction of RiCof. The plasma and platelet multimeric pattern show a relative reduction of the proportion of the largest multimers [4]. All these features were present in our family. Also the biological response to DDAVP was similar to that reported by Mannucci et al. [4], with no correction of the RiCof/vWF:Ag discrepancy and little or no BT shortening in the propositus and his son II.1 (Table II). Platelet counts were not obtained after DDAVP in the cases described by Mannucci et al. [4]. In our propositus and his son, thrombocytopenia occurred immediately after the end of infusion (Table II). Peripheral blood smears obtained at the end of infusion showed the presence of platelet clumps. This finding is usually observed in type IIB vWD [3] in which abnormal vWF is released into the circulation by DDAVP and, due to its enhanced affinity for glycoprotein Ib on platelet membrane, causes platelet clumping [3]. However, type IIB vWD can be easily ruled out in our family. RIPA occurs at low ristocetin concentrations in type IIB [3,10] whereas it occurred at normal or slightly increased concentrations in our patients. All multimers were detectable in the plasma of this patient, contrasting with the absence of larger multimers in type IIB patients [10]. Platelet vWF multimers are usually normal in type II B [11], whereas in our case larger multimers were reduced as in plasma. Proteolysis of plasma vWF, increased in type IIB [12], was normal in our case. The binding of plasma vWF to normal platelets occurred at normal ristocetin concentrations, whereas it occurs at lower concentrations in type IIB [10]. The binding of plasma vWF to normal platelets in the presence of botrocetin paralleled that observed with ristocetin (not shown).

The reasons for thrombocytopenia in our cases are not clear. Since the in vitro binding of vWF to glycoprotein Ib promoted by ristocetin or botrocetin is not increased, a heightened affinity of an abnormal vWF for a different platelet receptor might be postulated. Further studies are needed to elucidate the molecular mechanisms responsible for the post-DDAVP thrombocytopenia in type I vWD, subtype platelet discordant.

REFERENCES

1. Rodeghiero F, Castaman G, Mannucci PM: Clinical indications for desmopressin in congential and acquired von Willebrand disease. Blood Rev 5:155, 1991.

2. Rodeghiero F, Castaman G, Meyer D, Mannucci PM: Replacement therapy with virus-inactivated plasma concentrates in von Willebrand disease. Vox Sang, 62:193, 1992.
3. Holmberg L, Nilsson IM, Borge L, Gunnarson M, Sjorin E: Platelet aggregation induced by 1-desamino-8-D-arginine vasopressin (DDAVP) in type IIB von Willebrand's disease. N Engl J Med 309:816, 1983.
4. Mannucci PM, Lombardi R, Bader R, Federici AB, Solinas S, Mazzucconi MG, Mariani G: Heterogeneity of type I von Willebrand's disease: Evidence for a subgroup with an abnormal von Willebrand factor. Blood 66:796, 1985.
5. Rodeghiero F, Castaman G, Tosetto A: von Willebrand factor antigen is less sensitive than ristocetin cofactor for the diagnosis of type I von Willebrand disease—Results based on an epidemiological investigation. Thromb Haemostas 64:349, 1990.
6. Rodeghiero F, Castaman G, Dini E: Epidemiological investigation of the prevalence of von Willebrand's disease. Blood 69:454, 1987.
7. Mannucci PM, Lombardi R, Castaman G, Dent JA, Lattuada A, Rodeghiero F, Zimmerman TS: von Willebrand's disease "Vicenza" with larger-than-normal (supranormal) von Willebrand factor multimers. Blood 71:65, 1988.
8. Rodeghiero F, Castaman G, Di Bona E, Ruggeri M, Lombardi R, Mannucci PM: Hyper-responsiveness to DDAVP for patients with type I von Willebrand's disease and normal intra-platelet von Willebrand factor. Eur J Hematol 40:163, 1988.
9. Rodeghiero F, Castaman G, Tosetto A, Lattuada A, Mannucci PM: Platelet von Willebrand factor assay—Results using two methods for platelet lysis. Thromb Res 59:259, 1990.
10. Ruggeri ZM, Pareti FI, Mannucci PM, Ciavarella N, Zimmerman TS: Heightened interaction between platelets and Factor VIII/von Willebrand Factor in a new subtype of von Willebrand's disease. N Engl J Med 302:1047, 1980.
11. Ruggeri ZM, Zimmerman TS: Variant von Willebrand disease. Characterization of two subtypes by analysis of multimeric composition of factor VIII/von Willebrand factor in plasma and platelets. J Clin Invest 65:318, 1980.
12. Zimmerman TS, Dent JA, Ruggeri ZM, Nannini LH: Subunit composition of plasma von Willebrand factor. Cleavage is present in normal individuals, increased in II A and II B von Willebrand disease, but minimal in variants with aberrant structure of individual multimers (type II C, II D, II E). J Clin Invest 77:947, 1986.