American Journal of Hematology 49:83–86 (1995)

Characterization of Two Cases of Acquired Transitory von Willebrand Syndrome With Ciprofloxacin: Evidence for Heightened Proteolysis of von Willebrand Factor

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We characterized the cause of two cases of transitory acquired von Willebrand syndrome associated with the administration of ciprofloxacin. Purified Ig from the two patients did not inhibit Ristocetin Cofactor activity or binding to collagen of normal plasma, ruling out the possibility of an inhibitor. The analysis of multimeric pattern of plasma von Willebrand Factor (vWF) showed the lack of larger multimers in both patients, with a relative decrease of all the remaining forms in the first patient. The subunit composition of plasma vWF showed a marked reduction of the native 225 Kd subunit (31.9% and 32.9%; normal range 74–86%) and an increased proportion of the 189, 176, and 140 Kd fragments. These abnormalities disappeared during the follow-up, without any specific therapy. In conclusion, a common pathophysiological basis is demonstrated in both patients, with a heightened proteolysis of plasma vWF by an unknown mechanism. © 1995 Wiley-Liss, Inc.

Key words: von Willebrand factor, acquired von Willebrand syndrome, bleeding time, ciprofloxacin, acquired bleeding disorder

INTRODUCTION

The acquired von Willebrand syndrome (avWS) has been described in association with several hematological and nonhematological disorders [for review, see 1]. Among the pharmacological compounds, only valproic acid has been reported to be clearly associated to avWS [2]. We briefly described two cases of transitory avWS possibly associated with the administration of ciprofloxacin [3]. In this paper, we report extensive investigations in order to characterize the causes underlying this disorder.

MATERIALS AND METHODS

Case Report

The two patients had abrupt onset of bleeding symptoms shortly after the intake of ciprofloxacin. A diagnosis of acquired von Willebrand syndrome was made on the basis of low F VIII/von Willebrand factor (vWF) measurements and prolonged bleeding time (BT). Close monitoring showed that the disorder spontaneously disappeared over a 5 month period in both patients, without any specific therapy.

Methods

Blood was collected into 3.8% sodium citrate and centrifuged at 2,500g for 15 min for plasma analysis. Fibrinogen and D-dimer levels were determined as previously described [4]. Platelet count was carried out on EDTA-anticoagulated blood on Coulter Counter IV Plus (Hialeah, FL). Factor VIII procoagulant activity (VIII:C), von Willebrand factor antigen (vWF:Ag), Ristocetin cofactor activity (RiCof), and BT were determined as previously described [5,6].

Plasma for multimeric analysis of von Willebrand factor and subunit composition was collected in a cocktail of sodium citrate (0.129 mmol), EDTA (5 mmol), N-ethyl-

Received for publication August 5, 1994; accepted December 12, 1994.

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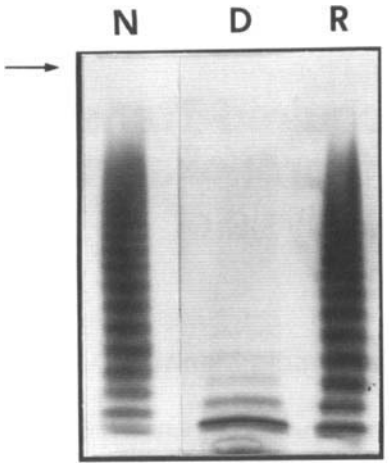
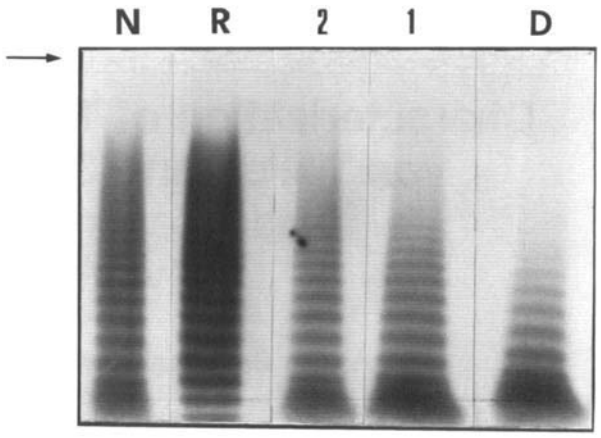


VIII:C (IU/dL)	8	78
vWF:Ag (IU/dL)	5	109
RiCof (IU/dL)	<3	88

Fig. 1. Multimeric pattern of plasma von Willebrand factor in patient 1. D, diagnosis; R, 5 months later (remission); N, normal plasma. The arrow at the top indicates the interface between stacking and running gel.



VIII:C (IU/dL)	88	58	69	45
vWF:Ag (IU/dL)	91	43	26	22
RiCof (IU/dL)	82	31	20	9

Fig. 2. Multimeric pattern of plasma von Willebrand factor in patient 2. D, diagnosis; 1, 1 month later; 2, two months later; R, 5 months later (remission); N, normal plasma. The arrow at the top indicates the interface between stacking and running gel.

maleimide (6 mmol), and leupeptin (1 mmol). The multimeric composition of vWF was analysed by sodium dodecyl sulphate (SDS)-agarose gel electrophoresis in low-resolution (0.9% low-gelling temperature agarose) gel system [7]. The subunit composition of vWF was analysed by immunoblotting with anti-vWF monoclonal antibodies and ^{125}I-rabbit anti-mouse antibody. Relative concentrations of vWF fragments were determined by cutting out radioactive bands from the nitrocellulose blot and quantitating radioactivity in a gamma scintillation counter [8].

Inhibitor Studies

vWF-binding assay. The IgG fraction of the patient and normal controls were purified by affinity chromatography on Sepharose-Protein A (Pharmacia, Uppsala, Sweden), as recommended by the manufacturer. Serially diluted Ig were incubated with a constant amount of normal plasma. Residual VIII/vWF activities were then measured on the supernatant of the adsorbed plasma.

Inhibition of vWF binding to collagen. Binding of normal and patient plasma vWF to collagen, with and without normal and patient purified Ig, was measured using an ELISA method, as previously described [9].

RESULTS

Figure 1 reports the multimeric pattern of plasma vWF in patient 1 along with VIII/vWF and BT measurements at presentation and at the time of normalization of plasma parameters. VIII/vWF measurements were severely reduced at admission. The intensity of vWF multimers was markedly reduced and the larger forms undetectable. At the time of normalization of plasma VIII/vWF measurements, a normal multimeric pattern was observed.

Figure 2 reports the multimeric pattern of plasma vWF in patient 2 along with VIII/vWF and BT measurements at presentation and during the follow-up. At presentation, the larger and intermediate multimers were lacking. During the follow-up, these forms gradually reappeared with the normalization of the pattern 5 months later.

Table I summarizes the results of the vWF subunit analysis on admission and on remission 5 months later. A heightened proteolysis of the native 225 Kd subunit is evident, with major increase of all the naturally occurring fragments. No novel bands were evident. Five months later, a normal relative distribution of the fragments was observed, with only a slight increase of the 176 kd fragment. In patient 2 it was possible to demonstrate the time-course normalization of subunit pattern (Table I).

At admission, platelet count and fibrinogen level were normal in both patients (not shown). D-dimer level was below 200 ng/mL.

Inhibitor studies failed to demonstrate the presence of autoantibodies with specific inhibiting activities (data not shown).

DISCUSSION

Recently, ciprofloxacin has been associated to several adverse effects, possibly having a common immune-re-

TABLE I. Subunit Composition of Plasma von Willebrand Factor at Diagnosis and 5 Months Later

	Relative percentage of the fragments (%)			
	225 Kd	189 Kd	176 Kd	140 Kd
Patient 1				
Diagnosis	31.9	27.3	16.6	24.2
Remission	78.7	6.9	8.9	5.5
Patient 2				
Diagnosis	32.9	26.3	20.6	20.2
1 month	35.5	14.1	26.7	23.7
4 months	64.4	6.5	16.9	12.2
Remission	79.4	7.1	8.8	4.7
Normal range	74–86	1.9–5.8	5.5–12.6	4–8

lated aetiology [10–15]. In particular, a short-lived inhibitor against factor VIII has been reported in a patient with hemophilia A [16]. Over a 1 year period, we observed two previously healthy subjects who developed avWS strongly suspected to be associated to ciprofloxacin [3]. However, no evidence for immune-related aetiology was found in our cases and no inhibitor against RiCof activity or collagen cofactor of vWF was detectable.

The analysis of plasma vWF multimeric pattern demonstrated the lack of the larger and intermediate size multimers. This abnormality was associated with a marked reduction of the native 225 kd subunit of vWF and increase of the other three naturally occurring fragments of 189, 176, and 140 kd. Thus, enhanced proteolysis of vWF was evident in both patients, producing a similar pattern of abnormality in vWF multimers and a remarkably similar clinical picture. In one patient, a progressive normalization of the subunit pattern was evident. There are several acquired conditions in which vWF may be proteolyzed. These include disseminated intravascular coagulation [17], pancreatitis, liver cirrhosis, acute promyelocytic leukemia [18], myeloproliferative syndromes [19], or thrombolytic therapy [20]. None of our patients had similar features. Moreover, fibrinogen, platelet count and D-dimer level were normal, thus ruling out the presence of low-grade intravascular clotting or of primary fibrinogenolysis. Hence, in our patients the factor(s) responsible for the observed abnormalities remain obscure.

To our knowledge, avWS has been associated only to the use of valproic acid [2]. In the 30 children evaluated, these authors found ten cases with low vWF and/or prolonged BT associated to a mild bleeding tendency. At variance with our cases, in Kreuz' cases [2] VIII/vWF measurements and multimeric analysis gave substantial evidence for a type I vWD pattern. It is relevent that the abnormality in our cases lasted about 5 months, despite the prompt discontinuation of the drug. The reason for such a sustained effect remains unclear.

In conclusion, we have demonstrated a heightened, long-lasting proteolysis of vWF in two patients taking ciprofloxacin, leading to an acquired bleeding diathesis. Coagulation studies are advisable in patients developing bleeding symptoms after or during ciprofloxacin assumption.

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