CONCISE REPORT

In Vivo Interaction of von Willebrand Factor With Platelets Following Cryoprecipitate Transfusion in Platelet-Type von Willebrand’s Disease

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Previous studies performed in vitro have indicated that platelets from patients with platelet-type von Willebrand’s disease (vWD) have receptors for von Willebrand factor (vWF) already exposed on their surfaces and that the addition of purified vWF or cryoprecipitate to patient platelet-rich plasma under stirring conditions is capable of inducing platelet aggregation and secretion. The present work reports the results of the transfusion of cryoprecipitate in a patient with platelet-type vWD. It is shown that, while factor VIII-related antigen and ristocetin cofactor activities maintain elevated levels for up to 12 hr following transfusion, the highest molecular weight vWF multimers decline rapidly. The platelet count also declines, followed in turn by a rise in the plasma level of platelet factor 4. Shortening of the bleeding time occurs only very transiently. The results of this study provide direct evidence that, in patients with platelet-type vWD, an abnormal interaction of their platelets with plasma vWF occurs in vivo, resulting in the absence of high molecular weight vWF multimers, low platelet counts, and impaired hemostasis that are characteristic of this disease.

WE HAVE RECENTLY DESCRIBED a family with an autosomally transmitted bleeding disorder, termed platelet-type von Willebrand’s disease (vWD), in which an absence of the higher molecular weight multimers of von Willebrand factor (vWF) in patient plasma occurs in conjunction with an increased ability of patient platelets to bind vWF and to aggregate in the presence of low concentrations of the antibiotic, ristocetin.1 In type IIB vWD there is also a decrease of the larger vWF multimers2 and an increased ristocetin-induced aggregation of platelet-rich plasma (PRP) in affected patients.3 However, we have now shown that in platelet-type, but not type IIB, vWD the addition of normal plasma cryoprecipitate or purified vWF to patient PRP is capable of inducing aggregation, and that 125I-vWF binds to unstimulated patient platelets in a specific and saturable manner.4 Although the patients that we have studied, as well as those with apparently similar disorders studied by others,5,6 tend to have generally mild bleeding diatheses characterized by epistaxis and mucocutaneous bleeding, some of these patients have occasionally had more serious bleeding episodes, particularly in conjunction with surgical procedures or trauma. Since the higher molecular weight vWF multimers, which are thought to be particularly important for the interaction of vWF with platelets,7,8 are absent from the plasma of these patients, attainment of higher plasma levels of these multimers would appear likely to be a necessary condition for adequate surgical hemostasis. Due to the ability of the patients’ own platelets to bind vWF in the absence of additional stimuli, the achievement of this goal in patients with platelet-type vWD appears less straightforward than in any of the previously described vWD variants. The present studies reporting the transfusion of exogenous vWF in platelet-type vWD indicate that the infused normal vWF interacts with patient platelets in vivo, resulting in platelet activation and rapid loss from plasma of the highest molecular weight vWF multimers.

MATERIALS AND METHODS

The patient was a 26-yr-old white male previously diagnosed as having platelet-type vWD (patient II-3, ref. 1). His past history included frequent episodes of epistaxis, as well as profuse bleeding following dental extraction in the past year. The infusion of cryoprecipitate in this patient was performed as a therapeutic trial during hospitalization for the anticipated surgical repair of a herniated intervertebral disc. The patient had not taken any medications for the previous 10 days.

Twenty single donor units of cryoprecipitate were infused over a period of 45 min. Bleeding times prior to and following the cryoprecipitate infusion were performed on the forearm, using the single blade Simplate technique (General Diagnostics, Morris Plains, NJ). For coagulation and platelet function studies, blood was drawn at the indicated times by a two-syringe technique. Plasma not immediately assayed was flash-frozen and stored at -70°C until used. Platelet counts were performed on EDTA-anticoagulated blood or on citrated PRP with a Coulter S Plus (Coulter Electronics, Hialeah, FL). Blood for platelet factor 4 (PF4) radioimmunoassay was collected and the assay performed according to the manufacturer’s instructions (Abbott Laboratories, Chicago, IL).

Prothrombin time, activated partial thromboplastin time, and thrombin time measurements were performed by standard methods.
Baseline hemostatic parameters in this patient with previously diagnosed platelet-type vWD were most notable for a prolonged Simplate bleeding time (>20 min) and decreased ristocetin cofactor activity (10%). Coagulation screening tests, factor VIII:C, and factor VIII-related antigen activity were all within normal limits. The platelet count was near the lower limit of normal (148,000/μl).

Twenty single donor bags of cryoprecipitate, containing a total of approximately 2,200 U factor VIII:C, were infused over a period of 45 min. The transfusion was not accompanied by any untoward clinical events, although mild flushing around the anterior neck was transiently observed. Following transfusion, equal aliquots from the residual material in each cryoprecipitate bag were pooled, flash-frozen, and subsequently assayed. Ristocetin cofactor and factor VIII-related antigen activities of the pool were 860% and 770%, respectively, while VIII:C activity measured 430%.

Within 15 min following the cryoprecipitate infusion, the patient’s ristocetin cofactor activity had normalized (Fig. 1, Bottom). The ristocetin cofactor activity reached its peak value at 4 hr and did not fall below 100% activity throughout the 12 hr of this study. The factor VIII-related antigen and VIII:C activity reached their peak values at 2 hr and both remained elevated throughout the 12 hr.

Bleeding time decreased to 14 min halfway through the cryoprecipitate infusion (i.e., after the first 10 bags). Fifteen minutes following completion of the infusion the bleeding time was 12 min, but then returned to greater than 20 min at the 1-hr time point, where it remained for the duration of the study (Fig. 1, Top). A blood sample drawn halfway through the infusion showed a platelet count of 120,000/μl. The platelet count continued to fall following completion of
the cryoprecipitate infusion, reaching its lowest value (70,000/μl) by 1 hr postinfusion (Fig. 1, Top). This value remained essentially unchanged through the fourth hour postinfusion. The transfusion of 10 random donor units of platelet concentrates 5 hr following the cryoprecipitate elevated the platelet count to nearly 100,000/μl. The bleeding time was not measurably altered by the platelet transfusion.

Multimeric analysis of the preinfusion plasma (Fig. 2, extreme left lane) confirmed the presence of intermediate multimers but absence of the highest molecular weight vWF multimers in this patient. By 1 hr following cryoprecipitate infusion, a normal multimeric pattern had been achieved. The very largest multimers appeared to have already begun decreasing by 2 hr and were clearly absent by 4 hr. Several bands, representing multimers of higher molecular weight than were observed in the preinfusion plasma, remained in the patient’s plasma for at least 12 hr following cryoprecipitate transfusion.

The prothrombin time, activated partial thromboplastin time, and thrombin time remained within normal limits and essentially unchanged throughout this study. Fibrinogen degradation products never exceeded 2.5 μg/ml. Baseline plasma level of PF4 was mildly elevated at 27 ng/ml (normal range 0–20 ng/ml). For the first hour following cryoprecipitate infusion, there was no change in the plasma level of PF4, but by 2 hr this value had risen to 54 ng/ml (Fig. 3). It declined only slightly over the next 2 hr.

Prior to cryoprecipitate transfusion, the patient’s PRP showed aggregation in response to low (0.3 mg/ml) concentrations of ristocetin. The in vitro addition of cryoprecipitate (1 U/ml ristocetin cofactor activity, final concentration) also produced aggregation. Normal aggregation patterns were seen with the additions of epinephrine or calcium ionophore A23187.

Addition of the preinfusion PRP to the cuvette and commencement of stirring in the aggregometer (i.e., without the addition of any aggregating agent) resulted in less than 5% aggregation (Fig. 4, trace A). Addition of the 1-hr postcryoprecipitate infusion PRP to the aggregometer cuvette, in contrast, resulted in “spontaneous” aggregation (Fig. 4, trace B). By 2 hr following the cryoprecipitate infusion, the degree of “spontaneous” in vitro aggregation had largely normalized (Fig. 4, trace C).
Fig. 4. "Spontaneous" platelet aggregation resulting upon addition to the aggregometer cuvette of citrated platelet-rich plasma (PRP) prepared from blood samples obtained prior to (A), 1 hr following (B), and 2 hr following (C) the transfusion of 20 single donor units of cryoprecipitate to a patient with platelet-type vWD. Platelet counts in the PRP were 175,000/μl for A and 133,000/μl for both B and C. The cuvette was stirred at 1,200 rpm and maintained at 37°C. PPP, platelet-poor plasma.

DISCUSSION

This study confirms our previous conclusions, based on in vitro experiments,\textsuperscript{1,4} that hemostasis is impaired in patients with platelet-type vWD due to an abnormal interaction of their platelets with plasma vWF. The immediate decrease in platelet count and rapid clearance of the highest molecular weight vWF multimers following cryoprecipitate transfusion accordingly appear to be in vivo reflections of the ability of vWF to bind to these platelets in the absence of additional platelet stimuli.\textsuperscript{4} This immediate decrease, together with the failure of plasma PF4 to rise until 2 hr following vWF infusion, suggests that platelets having bound vWF might initially become adherent at sites within the vasculature prior to their full activation and consumption. Moreover, the initiation of platelet aggregation with rapid stirring in the aggregometer cuvette in the 1 hr PRP sample (Fig. 4, trace B) emphasizes the role that local hemodynamic conditions are likely to play in this process. It is of additional interest that, throughout the course of this study, no evidence was obtained suggesting activation of either the coagulation or fibrinolytic systems.

The rapid clearance of the higher molecular weight multimers following transfusion of exogenous vWF stands in contrast to the considerably slower clearance recently reported in the type IIA and IIB vWD variants.\textsuperscript{13} This difference provides further support for the conclusion that platelets from patients with platelet-type vWD are unique in having receptors for vWF already exposed on their surfaces.

Shortly after completion of the cryoprecipitate infusion, plasma activities of the individual components of the factor VIII/vWF complex increased to anticipated levels. Over the next several hours, however, plasma levels continued to rise (Fig. 1), suggesting the possibility of an endogenous contribution to the observed total rise.

At 1 and 2 hr following cryoprecipitate infusion, the larger vWF multimers appeared present in higher plasma concentration than at 15 min following the cryoprecipitate infusion (Fig. 2). The shortened bleeding time observed at the 15-min time point, however, had returned to the prolonged baseline level by 1 hr following cryoprecipitate infusion. This discordance raises the possibility that, in addition to restoration of normal plasma values of the larger vWF multimers, correction of additional, possibly platelet-related, abnormalities may have been required for sustaining adequate hemostasis in this patient.

The rise in the platelet count in response to platelet transfusion at 5 hr following cryoprecipitate infusion (Fig. 1) was only about half that anticipated. It is possible that some of the transfused platelets were themselves involved in an ongoing platelet-consuming process. The continued prolongation of the bleeding time, however, likely reflects, at least in part, the appreciable decline of the higher molecular weight vWF multimers that had occurred by this time (Fig. 2). Persistence of at least normal ristocetin cofactor levels provides further evidence that, unlike the vWF activity required for maintaining normal bleeding...
times, that required for subserving platelet interactions mediated by ristocetin persists in the intermediate multimeric forms.3

The attainment of a level of hemostasis adequate for major surgery remains a challenging problem in patients with platelet-type vWD. Possibly, smaller amounts of exogenous cryoprecipitate transfused at optimal intervals might succeed in raising the high molecular weight vWF multimers to levels promoting shortened bleeding times, without an unacceptable degree of binding to, and subsequent consumption of, patient platelets. Elevation of endogenous vWF levels through the administration of 1-deamino-8-D-arginine vasopressin (DDAVP) represents another possible therapeutic approach. In a patient described as having a variant of vWD that most likely represents platelet-type vWD, the intravenous administration of DDAVP appeared to provide adequate hemostatic coverage for dental extraction, although the transiently normalized bleeding time was again markedly prolonged by 2 hr postinfusion.6,14 The vWF binding properties of the abnormal patient platelets may accordingly prove to be the limiting factor for the attainment of surgical hemostasis, whether the elevation of plasma vWF is attempted by either an exogenous or endogenous approach.

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