Hemostatic Effect of Normal Platelet Transfusion in Severe von Willebrand Disease Patients

By Ricardo Castillo, Juan Montesagudo, Ginés Escolar, Antonio Ordinas, Manuel Magallón, and José Martín Villar

Platelet von Willebrand factor (vWF) has been suggested to play an important role in the hemostatic process. Clinical and experimental data indicate that bleeding time (BT) and platelet-vascular wall interaction cannot be normalized unless the defect of platelet vWF is also corrected. We have examined the effect of normal platelet concentrate transfusion 1 hour after cryoprecipitate infusion in five type III von Willebrand disease (vWD) patients. The cryoprecipitate infusion attained normal circulating levels of ristocetin cofactor, vWF antigen, and factor VIII activity. In two patients, cryoprecipitate infusion did not modify the BT (>30 minutes), whereas in the remaining three patients BT was only partially corrected (from >30 to 12, 18, and 21 minutes). However, the immediate platelet transfusion completely corrected the BT in four cases, and in one case it shortened the BT to 8.30 minutes (n = 3 to 8 minutes). In the perfusion study, cryoprecipitate infusion only resulted in a slight increase in platelet deposition (surface coverage range: 2.4% to 11.3%), whereas the platelet concentrate transfusion elicited a more marked improvement (range: 8.2% to 26.4%; P < .02 v postcryoprecipitate). These results suggest an important in vivo role of the platelet vWF in supporting platelet-vascular wall interaction. They also give support to the occasional addition of normal platelet transfusion to the cryoprecipitate infusion for the control of serious bleeding episodes resistant to cryoprecipitate in severe vWD patients.

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PLASMA von Willebrand factor (vWF) is necessary for the normal platelet adhesion to the damaged vessel wall. There are several suggestions that the vWF present in the platelet α-granules plays a role in the hemostatic process as well. It has been observed that type I von Willebrand disease (vWD) patients with normal levels of platelet vWF, have shorter bleeding times (BT) than those with low or dysfunctional platelet vWF, despite equally low plasma vWF. Pigs with severe vWD engrafted with normal bone marrow, producing vWF-containing platelets, had shorter BT than before transplantation, despite the fact that plasma vWF levels remained very low. Recently, it has been shown that the transfusion of plasma concentrates to type III vWD patients is not able by itself to correct the BT even though both the vWF activities and the multimeric structure of vWF have been normalized.

In vitro perfusion assays have also shown that the adhesion to collagen of platelets from type III vWD (containing no measurable vWF) resuspended in normal plasma was much lower than that of normal platelets. Similar results were obtained using platelets from type III or type II vWD patients (containing dysfunctional vWF) and rabbit subendothelium as an adhesive surface. To evaluate the in vivo role of platelet vWF in the hemostatic process, we have studied, in severe vWD patients, the effect of normal platelet transfusion, performed following cryoprecipitate infusion, on the BT and the platelet adhesion to the subendothelium.

MATERIALS AND METHODS

Patients. Five type III vWD patients, with severe bleeding tendency, were included in the study on the occasion of macousal haemorrhage that needed replacement therapy. The study was approved by the Hospital Committee on Human Experimentation. Informed consent was obtained in each case according to the Declaration of Helsinki. Markers for hepatitis B were present in all patients before the assay. All patients presented HLA alloimmunization, and human immunodeficiency virus (HIV) antibodies were detected in four. All patients had previously shown in many occasions a poor clinical response to cryoprecipitates.

All patients consistently had no vWF ristocetin cofactor (vWF: RCo) in plasma, no measurable vWF antigen (vWF:Ag) in plasma and platelets, factor VIII activity (VIII:C) levels in plasma less than 6 U/dL, and presented a BT longer than 30 minutes. None of them had any inhibitor activity against vWF at the time of the assay. Patients' weight ranged from 56 to 80 kg, and they were between 18 and 57 years old.

Design of the study. Cryoprecipitate was infused to patients over a period of 30 minutes after having determined the BT; the plasma levels of vWF:RCo, vWF:Ag, and VIII:C; the platelet vWF:Ag; and the platelet interaction on the vessel wall. The dose administered was tailored to attain levels of those plasmatic activities of at least 100 U/dL.

One hour after the end of the cryoprecipitate infusion, normal platelet concentrate was transfused over a period of approximately 20 minutes. The mean platelet number transfused to each patient was 3.9 ± 0.7 × 10^11. Measurements of the BT; of the plasma levels of vWF:RCo, vWF:Ag, and VIII:C; and of the platelet vWF:Ag had been repeated 1 hour after the beginning of the cryoprecipitate infusion, as well as 1, 4, and 16 hours after the beginning of the platelet transfusion. In one case the measurements at 4 and 16 hours were not determined. Platelet interaction on the vessel wall was studied before and after the cryoprecipitate infusion and 1 hour after the beginning of platelet transfusion. The multimeric structure of vWF was assessed in the infused cryoprecipitates, in all the plasma samples of patients, and in platelets of the concentrates transfused.

Blood fraction concentrates. Cryoprecipitate was prepared by the Hospital Blood Bank from single donations of fresh frozen plasma. It was stored at −80°C and units were pooled in plastic bags before infusion. Platelet concentrates were collected by apheresis, from a single HLA matched donor, using the Haemonetics V50-I surge pump method (Haemonetics Co, Braintree, MA). Both cryoprecipitates and platelet concentrates were tested for...
hepatitis B surface antigen (HbsAg), serum transaminases, and HIV antibodies.

Methods. BT was measured using the "Simplate" sterile disposable device (General Diagnostics, Morris Plains, NJ). A blood pressure cuff was inflated to 40 mm Hg pressure and two vertical incisions 6-mm long and 1-mm deep were made on the volar surface of the forearm. Results were expressed as the average BT of the two incisions. All BT were measured by the same experienced operator. The normal range was 3 to 8 minutes. The expression of the BT was similar as previously described: it was defined as "corrected" or "normalized" when it became shorter than or equal to 8 minutes, our upper normal limit for this test; it was defined as "partially corrected" when it shortened from baseline values (more than 30 minutes for all patients) by at least 30% (to 21 minutes or less), this value being the between-assay coefficient of variation of the method; finally, it was defined as "unchanged" when it remained 21 minutes or longer.

Blood samples were collected from antecubital veins into one-tenth volume of 129 mmol/L trisodium citrate. Platelet-poor plasma was obtained after centrifugation (2,800g for 20 minutes) at 4°C and immediately stored at −80°C until tested. Plasma VIII:C was assayed by the one-stage kaolin activated partial thromboplastin time method. Plasma vWF:Ag was measured by an enzyme-linked immunosorbent assay (ELISA) method and plasma vWF:RCo by using formaldehyde-fixed platelets as previously reported. The platelet vWF:Ag was also measured by ELISA in washed platelets lysed by 0.1% Triton X-100 (Sigma, St Louis, MO). The multimeric structure of vWF in plasma and in platelet concentrates was analyzed by sodium dodecyl sulphate (SDS) agarose gel electrophoresis.

Platelet interaction with the vessel wall was studied in the perfusion system developed by Baumgartner and Muggli using citrated whole blood and subendothelium of rabbit abdominal aortic segments. Perfusions were performed at 37°C through the Baumgartner plastic annular chamber with a width of 0.45 mm. The flow was established by pumping through a hemodialysis pump (Renal Systems, Minneapolis, MN) and the flow rate was fixed at 0.2 mL/min (shear rate: 1,300 sec⁻¹). The whole blood was circulated through the system for 10 minutes. The endothelium of the vessel segments was removed by brief air exposure followed by rinsing with flowing phosphate buffered saline (PBS), pH 7.4. Evaluation of the interacting platelets was performed according to the overall deposition of platelets on vascular subendothelium (CS), and to the platelet aggregates extending more than 3 μm into the lumen (Ag). Details of the procedure and morphometric analysis were as previously described. Wilcoxon's test for paired data was used to compare the differences between the levels of surface coverage by platelets before and after normal platelet transfusion.

RESULTS

vWF. The average of the vWF:RCo infused with the cryoprecipitates was 64.7 ± 20.1 U/kg and the average of the levels attained in the patient's plasma 1 hour after the beginning of the infusion was 102.0 ± 12.9 U/dL, which subsequently declined. Levels were found to be above 50 U/dL in all five patients for more than 5 hours after the beginning of the cryoprecipitate infusion, and in two of the patients levels remained above 50 U/dL for more than 17 hours (Fig 1). The immediate postinfusion levels and subsequent decrease of the vWF:Ag were similar to those of the vWF:RCo (average of the peak levels: 104.6 ± 7.7). The levels of VIII:C were slightly higher than those of the vWF:Ag and vWF:RCo (average of the peak levels: 120.1 ± 19.8). The vWF:Ag of the platelet concentrates was 0.44 ± 0.05 U/10⁹.

The vWF multimeric pattern was assessed in both reconstituted cryoprecipitates and isolated platelets from platelet concentrates by means of intermediate-resolution SDS-agarose gel electrophoresis. Multimeric size was normal in these cases, as well as in the patients' postinfusion plasmas. Higher-than-normal vWF multimers were not investigated by low-resolution gels.

The vWF:Ag levels in platelet lysates 1 hour after the beginning of the normal platelet transfusion in the five patients ranged between 0.015 and 0.061 × 10⁹ platelets (Fig 2). In all patients, the latter values were slightly increased 4 hours after transfusion and remained around these levels after 16 hours (normal range: 0.33 to 0.60 × 10⁹) (Fig 2).

BT. Figure 1 shows the changes of the BT before and after cryoprecipitate and platelet transfusions in the five patients studied. After cryoprecipitate infusion, the BT remained unchanged in two patients (>30 minutes) and partially corrected in three (17, 18, and 21 minutes). One hour after the beginning of the transfusion of platelet...
Concentrate, performed after cryoprecipitate infusion, the BT was normalized in four cases (3, 4, 5, and 8 minutes) and in one case it was shortened to 8.30 minutes (n: 3 to 8 minutes). At 4 hours, these levels remained corrected. After 16 hours, the BT in one patient remained corrected (6.30 minutes) and in the other three it was partially corrected (11, 13, and 15 minutes). The patient’s bleeding was not stopped until the BT was normalized. Cryoprecipitate infusion was not effective in mucosal bleeding. After platelet concentrate transfusion bleeding stopped in all patients (Table 1).

Platelet interaction with vessel subendothelium. The surface covered by platelets before any treatment was initiated was less than 3.4% in all five cases (mean normal levels: 21 ± 4%). One hour after cryoprecipitate infusion values of covered surface ranged from 2.4% to 11.3% and platelet aggregates were absent. One hour after platelet transfusion, the levels were significantly higher in all five patients (P < 0.02) than those attained after cryoprecipitate infusion, and aggregates appeared (Table 2).

### DISCUSSION

Several investigators have reported the failure of plasma concentrates to correct the BT and to control clinical hemorrhage in type III vWD patients, although the plasma levels of vWF and VIII:C activities attained normality.\(^4\) The lack of correction of the BT occurs even when the vWF multimeric structure is intact in the plasma concentrate (cryoprecipitate and fraction I-O) as well as in the corresponding postinfusion patients’ plasmas.\(^4\) Moreover, the normalization of BT after cryoprecipitate infusion, when achieved, does not remain for longer than 4 to 6 hours.\(^2\)\(^3\)\(^22\)\(^22\)

In this study, the infusion of cryoprecipitate did not correct the prolonged BT in type III vWD patients. However, BT were normalized after adding normal platelet transfusion. The patient’s response to cryoprecipitate infusion has been considered to serve as a control experiment on the effect of platelet transfusion. The results observed suggest the in vivo contribution of platelet vWF to the arrest of bleeding and support the exceptional use of normal platelet concentrates in severe vWD patients.

The attainment of a normal postinfusion BT by the Duke technique would be the most important predictor of a satisfactory hemostasis, as one group of investigators has claimed.\(^2\) A template device was used in our study. In fact, the number of BT tests required for each patient precluded the use of carboembol methods. To date, incision controlled by a template has been proved to be the most sensitive technique in assessing BT.\(^2\) Whether or not a normal BT should be attained in vWD to ensure a correct hemostasis is a question not completely settled. Early clinical studies indicate that, whatever the BT, soft-tissue and postoperative bleeding would be stopped provided that normal postinfusion VIII:C levels are attained,\(^3\)\(^4\)\(^28\) but the mucosal bleeding will not be controlled unless the bleeding time is normalized.\(^1\)\(^7\)\(^9\)

In the present study, platelet transfusion after cryoprecipitate infusion corrected the BT in all but one patient. However, this last case showed a borderline normal value (8.30 minutes; normal range: 4 to 8 minutes). In all patients, the duration of the normalization of the BT was up to 4 hours after the beginning of the platelet concentrate.

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### Table 1. Clinical Outcome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type of Bleeding</th>
<th>After CP</th>
<th>After PLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>△</td>
<td>Duodenal hemorrhage</td>
<td>Slight</td>
<td>Complete</td>
</tr>
<tr>
<td>▲</td>
<td>Metrorrhagia</td>
<td>Unchanged</td>
<td>Complete</td>
</tr>
<tr>
<td>○</td>
<td>Severe epistaxis</td>
<td>Unchanged</td>
<td>Complete</td>
</tr>
<tr>
<td>●</td>
<td>Metrorrhagia</td>
<td>Slight</td>
<td>Complete</td>
</tr>
<tr>
<td>□</td>
<td>Pneumothorax</td>
<td>Unchanged</td>
<td>Complete</td>
</tr>
</tbody>
</table>

Abbreviations: CP, cryoprecipitates; PLC, platelet concentrate.

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### Table 2. Platelet Interactions on Vessel Subendothelium

<table>
<thead>
<tr>
<th>Patients</th>
<th>Baseline</th>
<th>After Cryoprecipitate Infusion</th>
<th>After Platelet Transfusion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>△</td>
<td>1</td>
<td>2.8 ± 1.0</td>
<td>8.2 ± 3.1</td>
</tr>
<tr>
<td>▲</td>
<td>1</td>
<td>11.3 ± 3.6</td>
<td>26.4 ± 4.4</td>
</tr>
<tr>
<td>○</td>
<td>1</td>
<td>3.1 ± 0.1</td>
<td>8.7 ± 0.9</td>
</tr>
<tr>
<td>●</td>
<td>3.4 ± 0.2</td>
<td>10.3 ± 2.1</td>
<td>17.6 ± 1.2</td>
</tr>
<tr>
<td>□</td>
<td>1.4</td>
<td>2.4 ± 0.4</td>
<td>14.4 ± 0.1</td>
</tr>
</tbody>
</table>

Normal values: 21 ± 4.4

Values represent mean ± SD of three different perfusions for each blood sample. The presence (+) or absence (−) of platelet aggregates is indicated between brackets.

*One hour after the beginning of the platelet transfusion.
transfusion, and in one of four it was up to 16 hours. At this time the other three patients showed shorter BT than after cryoprecipitate infusion. This correction of the BT with normal platelet transfusion over such a period of time, after cryoprecipitate infusion, had not been previously reported in humans by means of cryoprecipitate treatment alone. Recently, Cattaneo et al.6 have observed the shortening of BT after the administration of 1-desamino-8-d-arginine vasopressin (DDAVP) in type III vWD after infusion of cryoprecipitates. Although the effect of DDAVP in this situation was somewhat less effective than that we observed with platelet transfusions, this is an interesting finding because it avoids the risks of platelet transfusions.

After treatment with cryoprecipitate, the improvement of the platelet interaction with subendothelium was low and platelet aggregates were absent. However, after normal platelet transfusion, the surface coverage by platelets was significantly increased versus the pretransfusion values and aggregates appeared. These results coincide with those of the BT.

The platelet dose transfused to each patient ranged from 3 x 10^11 to 6 x 10^11 platelets, being a dose that is usually administered to patients with amegakaryocytic thrombocytopenia and congenital qualitative platelet disorders. Both the platelet count (data not shown) and the levels of platelet vWF were slightly higher 4 hours after platelet transfusion than after 1 hour. This may be partially explained by a low initial platelet recovery, which has been related to a temporary sequestration of platelets in the spleen. The antibody assays, which were not sensitive enough to detect all the transfused platelet antibodies, as it is usual in polyclonally transfused patients, might have been an additional cause in the poor platelet recovery. Platelet concentrates were obtained from histocompatible and HLA-matched single donors to attain the maximum yield of transfused platelets. However, there is an indication of platelet concentrates after cryoprecipitate infusion in those situations where this last treatment will not control serious hemorrhages in severe vWD.

In conclusion, the results of this study suggest an important in vivo role of the platelet vWF in the hemostatic process, especially in vWD. They also support the clinical indication of platelet concentrates after cryoprecipitate infusion in those situations where this last treatment will not control serious hemorrhages in severe vWD.

ACKNOWLEDGMENT

The authors thank Dr P.M. Mannucci for his fruitful criticisms, Clara Pérez for preparation of the manuscript, and Marta Garrido for technical assistance.

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