Comparison of Four Virus-Inactivated Plasma Concentrates for Treatment of Severe von Willebrand Disease: A Cross-Over Randomized Trial

By Pier Mannuccio Mannucci, Paola M. Tenconi, Giancarlo Castaman, and Francesco Rodeghiero

Until recently, cryoprecipitate has been the treatment of choice in patients with severe von Willebrand disease (vWD) because it can transiently correct low plasma levels of factor VIII coagulant activity (FVIII:C) and shorten or normalize the prolonged bleeding time (BT), the two laboratory hallmarks of the disease. However, cryoprecipitate may still transmit blood-borne viruses, whereas the development of virucidal methods have rendered plasma concentrates containing FVIII:C and von Willebrand factor (vWF) safer. To establish their potential usefulness in the treatment of vWD, we compared the effect of four virus-inactivated concentrates on FVIII:C and vWF plasma levels and the BT (template method) in 10 patients with severe vWD using a crossover randomized design. The concentrates were an intermediate-purity, pasteurized FVIII-vWF concentrate; an intermediate-purity, dry-heated FVIII-vWF concentrate; a solvent/detergent-treated vWF concentrate, containing little FVIII; and a high-purity solvent/detergent-treated FVIII-vWF concentrate. All concentrates were equally effective in attaining normal and sustained levels of FVIII:C postinfusion, although peak levels were more delayed after the vWF concentrate. The effect of concentrates on the BT, however, was less uniform and satisfactory. The pasteurized FVIII-vWF concentrate transiently corrected, completely or partially, the BT in 8 of 10 patients, the dry-heated and solvent/detergent FVIII/vWF concentrates in five, whereas in no patient did the vWF concentrate correct the BT according to the criteria used in this study. These effects on the BT were not related to the plasma levels of ristocetin cofactor activity-attained postinfusion (100 U/dL or more in the majority of patients) or to the multimeric structure of vWF in concentrates (defective in larger multimers in all cases). In conclusion, even though virus-inactivated concentrates can be used to increase FVIII:C levels in patients with severe vWD, none of the concentrates studied by us consistently normalizes the BT in a sustained fashion.

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In von Willebrand disease (vWD), the main goals of treatment are to correct the deficiency of plasma factor VIII coagulant activity (FVIII:C) and the prolonged bleeding time (BT), reflecting the dual derangement of primary hemostasis and coagulation and the patient’s tendency to bleed. In the majority of patients, both goals can be achieved by administering the synthetic drug desmopressin (DDAVP), which is of proven clinical efficacy in preventing or stopping bleeding. This drug is inexpensive and safe, with no risk of transmitting blood-borne viruses. However, approximately 10% to 20% of patients with vWD respond poorly to DDAVP, such as those with type III (severe), type I ("platelet low" and "platelet discordant"), and some patients with type II. Furthermore, patients initially responsive to DDAVP may become refractory when repeated infusions are administered to maintain hemostasis over long periods of time (such as, for instance, after surgical procedures). Therefore, replacement therapy with plasma concentrates still has a role in the treatment of vWD.

For many years, cryoprecipitate has been the mainstay of replacement therapy in vWD because it can transiently normalize FVIII:C, shorten or normalize the BT, and stop or prevent clinical bleeding in many cases. However, because virucidal methods cannot be currently applied to cryoprecipitate produced by blood banks, this plasma fraction carries a small, but definite risk of transmitting blood-borne viruses, particularly when repeated infusions over time expose the recipients to a large number of different donors. Recently, the development of a variety of virucidal methods have rendered plasma concentrates containing FVIII:C and von Willebrand factor (vWF) safer. This has led several investigators to consider these concentrates as a possible alternative to cryoprecipitate in the treatment of vWD and to assess their ability to correct the FVIII:C and BT defects and clinical efficacy. The limits of these anecdotal studies are that concentrates were administered at different doses and not compared directly with each other; and that heterogenous vWD patients, of different type and severity, were studied. Thus, we chose to compare the effect on FVIII:C and BT of four virus-inactivated concentrates in 10 patients with severe vWD in a crossover randomized design. Patients with severe vWD are a homogeneous group of patients in whom DDAVP is not effective and therefore administration of plasma concentrates is needed for treatment.

MATERIALS AND METHODS

Design of the Study

Ten multitransfused patients with severe vWD were included in the study after informed consent was obtained and the experimental nature of the study was explained, including the associated risks. The study was approved by an internal review board. At the time of the study, no patient was bleeding and no replacement therapy had been administered in the last 15 days. Each patient was randomly assigned to a single infusion with one of the four concentrates and then crossed-over to a single infusion with the remaining three after at least 15 days. The time interval between the first and last concentrate ranged between 8 and 11 months.

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We elected to base the concentrate dosages on their content of ristocetin cofactor (Ricof) activity, because previous studies have indicated that postinfusion Ricof is the measurement that correlates better with BT shortening in treated vWD patients (although other studies have found no such correlation). After the Ricof was measured in the concentrate batches used in the study (Table 1), the dose administered to each patient was tailored to attain 1-hour postinfusion Ricof levels of more than 100 U/dL. This practice produced levels based on our previous data indicating that in patients with severe vWD the infusion of 1 U/kg of Ricof raises plasma levels in the recipient plasma approximately 2.0 to 2.5 U/dL. Each concentrate was administered by intravenous infusion over a period of 5 to 10 minutes and blood samples were taken for FVIII:vWF measurements and multimeric analysis 1, 3, 6, and 24 hours postinfusion. The BT was measured only before the infusion and at 1, 6, and 24 hours.

Patients

The diagnostic criteria used to diagnose severe (type III) vWD have been reported. All patients had a BT consistently longer than 30 minutes, and no measurable Ricof activity (<6 U/dL) and vWF antigen (vWFAg; <1 U/dL) in plasma and platelets, with FVIII:C levels ranging between 2 U/dL and 24 U/dL (normal values, 52 to 154 U/dL). No vWF multimer could be detected in preinfusion plasma by agarose gel electrophoresis followed by autoradiography (see below). All patients had been transfused on multiple occasions in the past with cryoprecipitate, commercial FVIII-vWF concentrates, and other blood products, were positive for serum antibodies to hepatitis B surface and core antigens and for hepatitis C virus (c100-3 antigen), but none was positive for antibody to the human immunodeficiency virus.

Concentrates

Hemate P (produced by Behringwerke, Marburg, Germany; called Humate P in the United States and distributed by Armour Pharmaceuticals, Collegeville, PA) is an intermediate-purity FVIII-vWF concentrate licensed in Italy for the treatment of hemophilia A and was purchased on the marketplace as a single lot (91243). It contains relatively little FVIII:C [20]. The virucidal method is based on the treatment of the concentrate with a mixture of a lipid solvent and a detergent. Alpha VIII (lot AB0001; produced by Alpha Therapeutic, Langen, Germany) is a solvent/detergent-treated high-purity FVIII-vWF concentrate licensed for the treatment of hemophilia A in the United States it is manufactured by Alpha Therapeutic Corporation (Los Angeles, CA) and called Profilate HP.

Laboratory Methods

The methods used to assay FVIII:C, vWF:Ag, and Ricof activity in patient plasma have been published. The only modification adopted subsequently is the replacement of washed platelets with formalin-fixed platelets in the assay of Ricof. Values were expressed in units per deciliter with reference to a pooled normal plasma calibrated against the 2nd International Standard for Factor VIII Related Activities in Plasma (87/718, National Institute for Biological Standard and Controls, Potters Bar, UK). The same assay methods were modified to assay FVIII:C, vWF:Ag, and Ricof in the reconstituted concentrates. The main modifications were the use of plasma from untreated patients with severe hemophilia or severe vWD to dilute concentrates to values similar to those contained in normal plasma for the assays of FVIII:C and vWF, respectively, and the use of the 4th International Standard for FVIII:C concentrate (88/804) to measure the FVIII:C content in the concentrates.

The BT was measured by making two vertical incisions on the forearm with a template method (Simplate II; General Diagnostic, Milan, Italy). When the incisions were still bleeding at 30 minutes, the BT was halted and recorded as longer than 30 minutes. The same lot of Simplate II was used and only two experienced technicians, who did not know the concentrate infused, performed the BT in Milano and Vicenza. A postinfusion BT was considered "corrected" when it become shorter than or equal to 7 minutes, the upper laboratory limit of this test in our laboratories; "partially corrected" when it was shortened from baseline values (more than 30 minutes for all patients) by at least 30% (to less than 21 minutes), this figure being the between-assay coefficient of variation of our method; and "not corrected" when it was 21 minutes or longer.

The multimeric structure of vWF in concentrates and postinfusion plasmas was analyzed by sodium dodecyl sulphate agarose gel electrophoresis using a low-resolution system that partially resolves large vWF multimers and allows the detection of defects in this region. Plasma and concentrate samples were run in the same gel and adjusted to the same concentration of vWF:Ag before electrophoresis. The multimeric structure of concentrates was also analyzed using a high-resolution gel system that does not resolves larger multimers in normal human plasma but resolves smaller multimers into five bands, with two subbands above and below a more intense central band. Concentrates were compared with a normal plasma run in the same gel and adjusted to the same concentration of vWF:Ag before electrophoresis.

Table 1. Factor VIII and vWF Levels Contained in Concentrates and Infused Into Patients

<table>
<thead>
<tr>
<th>Assayed in reconstituted concentrates (U/mL)*</th>
<th>FVIII:C</th>
<th>vWF:Ag</th>
<th>Ricof</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemate P</td>
<td>21-24-28</td>
<td>54-69-72</td>
<td>60-86-71</td>
</tr>
<tr>
<td>8Y BLP</td>
<td>18-26-29</td>
<td>59-74-83</td>
<td>31-35-37</td>
</tr>
<tr>
<td>Very high purity vWF</td>
<td>3-4-5</td>
<td>24-34-39</td>
<td>30-34-41</td>
</tr>
<tr>
<td>Alpha VIII</td>
<td>96-126-145</td>
<td>832-963-1112</td>
<td>240-256-301</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infused into patients (U/kg)</th>
<th>FVIII:C</th>
<th>vWF:Ag</th>
<th>Ricof</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemate P</td>
<td>24 ± 8</td>
<td>70 ± 10</td>
<td>66 ± 9</td>
</tr>
<tr>
<td>8Y BLP</td>
<td>47 ± 9</td>
<td>134 ± 12</td>
<td>64 ± 12</td>
</tr>
<tr>
<td>Very high purity vWF</td>
<td>19 ± 2</td>
<td>57 ± 8</td>
<td>57 ± 9</td>
</tr>
<tr>
<td>Alpha VIII</td>
<td>35 ± 3</td>
<td>272 ± 13</td>
<td>71 ± 6</td>
</tr>
</tbody>
</table>

*The volume of fluid used to reconstitute each bottle of concentrate was 30 mL for Hemate P, 10 mL for 8Y, 20 mL for very high purity vWF, and 4.6 mL for Alpha VIII.
†Mean ± SD.
before electrophoresis, some differences in the intensity of the bands were seen. However, it is clear that no concentrate had an intact multimeric structure, and that all lacked multimers of large molecular weight present in normal plasma. Each concentrate was also compared with normal plasma using a high-resolution gel system. Figure 2 shows that in plasma and concentrate the smallest multimers were resolved into five bands. For all concentrates, all the subbands, but particularly the most anodal one, were broader and more intense than the corresponding plasma subbands.

Statistical Analysis

Data were evaluated by analysis of variance for repeated measures (different concentrates) and for one factor (time intervals after concentrates). When statistically significant differences were found, concentrates were compared with the Student’s t-test for paired data. P values less than .05 were accepted as statistically significant. Correlations between the bleeding time and Ricof values were evaluated by calculating the Spearman correlation coefficient (p) for nonparametric data.

RESULTS

FVIII:C and vWF in Concentrates

Table 1 shows the content of FVIII:C, vWF:Ag, and Ricof activity (values of three separate assays) in the concentrates after reconstitution in the amount of fluid recommended by the manufacturers. For all concentrates, the concentrations of FVIII:C were lower than those of vWF:Ag and Ricof, particularly for the vWF concentrate and Alpha VIII (which contained the largest amount of either moiety). In Hemate P and the vWF concentrate, vWF:Ag and Ricof were similar, whereas for 8Y and Alpha VIII there was a relative excess of vWF:Ag over Ricof. Table 1 also shows that for each concentrate the doses of Ricof actually administered to patients were slightly different from the scheduled dose of 60 U/Kg, because doses were adjusted for optimum use of the concentrate bottles. The relationship between the dose of Ricof administered and the patient response was similar to that predicted from our previous publication17: for Hemate P, the mean increase in Ricof at 1 hour was 2.2 ± 0.6 U/dL per U/kg infused; for 8Y BLP, 2.1 ± 0.8; for vWF concentrate, 2.3 ± 0.4; and for Alpha VIII, 2.4 ± 0.5.

Figure 1 shows the multimeric pattern of vWF in the concentrates, compared with normal plasma run as control, using a low-resolution gel system. Despite the fact that samples were adjusted to the same concentration of vWF:Ag

Factor VIII and vWF in Patients

Table 2 shows the mean values of FVIII:C and vWF measurements before and after concentrate infusion.

At 1 hour postinfusion, FVIII:C increased to a similar degree and achieved normal levels in all patients after Hemate P, 8Y, and Alpha VIII. As expected, after the vWF concentrate it increased less than after the FVIII-vWF concentrates (P < .05). In the subsequent postinfusion time intervals (3, 6, and 24 hours), FVIII:C levels were sustained for Hemate P, 8Y, and Alpha VIII. For the vWF concentrate, there was a progressive increase above the levels measured at 1 hour, so that at 24 hours the between-concentrate differences in FVIII:C levels were no longer statistically significant (Table 2).

At 1 hour postinfusion, the average Ricof levels were greater than 100 U/dL after all concentrates, as expected (Table 2). Even though higher levels were attained after Alpha VIII than after the remaining concentrates, the difference was not statistically significant. At the subsequent postinfusion times, Ricof levels declined at approximately the same rate for all concentrates (Table 2).

In agreement with the larger amount measured in vitro, vWF:Ag increased significantly more than Ricof after Alpha VIII (P < .05 at 1, 3, and 6 hours), whereas for the
remaining concentrates postinfusion values of Ricof and vWF:Ag were not significantly different (Table 2).

The behavior of vWF multimers after concentrate infusion was homogeneous. At 1 hour postinfusion, the plasma multimeric pattern reproduced that seen in the concentrate in vitro, depending on the degree of in vitro defects. In the subsequent postinfusion times, there was some degree of fading of the bands, with no preferential clearance of multimers of particular size (not shown).

Effect on the BT

Hemate P. Figure 3 shows that at 1 hour postinfusion, the BT was corrected in three patients, partially corrected in five, and not corrected in two. At 6 hours, there was a tendency for the BT to lengthen. After 24 hours, the BT was longer than 30 minutes in all of the patients. Figure 3 also shows that in all patients Ricof reached 1-hour postinfusion levels of at least 70 U/dL and that in most patients it reached values of 100 U/dL or higher.

8Y. Figure 4 shows that at 1 hour postinfusion the BT was corrected in one patient, partially corrected in four, and not corrected in five. It lengthened again in all patients at 6 hours. Ricof increased to 100 U/dL or more in all but two patients (49 and 57 U/dL).

Very high purity vWF. Figure 5 shows that, at 1 hour postinfusion, in no patient was the BT even partially corrected according to our criteria, remaining 24 minutes or longer. In three instances, the BT was somewhat shorter at 6 hours than at 1 hour (from longer than 30 minutes to 30 minutes; from 30 to 17 and 24 to 11 minutes). Despite the poor effect of this concentrate on the BT, in all patients Ricof reached levels of 100 U/dL or higher.

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Table 2. Changes of Factor VIII and vWF Measurements After Infusion of Concentrates

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Time Postinfusion (h)</th>
<th>Concentrates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hemate P</td>
</tr>
<tr>
<td>FVIII:C (U/dL)</td>
<td>0</td>
<td>11 ± 8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>86 ± 30</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>87 ± 28</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>82 ± 31</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>77 ± 28</td>
</tr>
<tr>
<td>Ricof (U/dL)</td>
<td>0</td>
<td>&lt;6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>140 ± 45</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>113 ± 44</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>72 ± 24</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>30 ± 20</td>
</tr>
<tr>
<td>vWF:Ag (U/dL)</td>
<td>0</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>185 ± 94</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>158 ± 95</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>125 ± 94</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>40 ± 41</td>
</tr>
</tbody>
</table>

*Significantly different (P < .05) from the remaining concentrates at the same time points (analysis of variance for repeated measures).

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Fig 2. Multimeric structure of vWF in concentrates compared with normal plasma run in the same high-resolution gel. The five subbands into which smaller multimers are resolved are identified by horizontal dashes.
Fig 3. Changes in BT (top panel) and Ricof (lower panel) in 10 patients with severe vWD before and after Hemate P. The horizontal broken lines indicate the upper limits of normal laboratory ranges. Each patient is identified by a different symbol. The scale of the vertical axis is logarithmic, that of the horizontal axis is arbitrary.

Alpha VIII. Figure 6 shows that at 1 hour postinfusion, two BT were corrected, three partially corrected, and the remaining five not corrected. At 6 hours, there was a tendency for the BT to lengthen. In all patients, Ricof levels were 100 U/dL or higher at 1 hour postinfusion.

Cumulating the data obtained after all concentrates, there was no statistically significant correlation between the BT and Ricof levels at 1 and 6 hours (Spearman’s ρ, −0.15; P > .3).

DISCUSSION

This is the first randomized study that compares the effect of virus-inactivated concentrates on plasma FVIII:C and vWF and BT using a crossover design. The main findings are that all concentrates were effective in attaining normal levels of FVIII:C postinfusion, but that none normalized the BT consistently and in a sustained fashion.

The effect on FVIII:C of such concentrates as Hemate P, 8Y, and Alpha VIII was predicted, because they are manufactured for the treatment of hemophilia A and hence contain large amounts of FVIII:C. A sustained increase of FVIII:C was maintained for at least 24 hours, in variance with what happens in treated hemophiliacs in whom FVIII:C declines over the same period of time. This phenomenon, already well established for plasma and cryoprecipitate, is due to the large amounts of vWF contained in concentrates (Table 1). vWF acts as a carrier and stabilizer of FVIII:C, which is synthesized at a normal rate in vWD, but is rendered less stable in plasma and susceptible to proteolysis by the deficiency of vWF. After the vWF concentrate, FVIII:C behaved differently from the remaining concentrates. FVIII:C levels were lower at the earliest postinfusion times, as expected from the lower amount contained in the concentrate, but increased progressively thereafter due
The effect of concentrates on the BT was less satisfactory. The concentrate that gave the best results was Hemate P, which transiently corrected completely or partially the BT in eight patients. Our findings confirm the results obtained by other investigators using this concentrate in less homogeneous patients with less uniform protocol.7,9,8Y and Alpha VIII gave complete or partial correction in five patients. In a previous study,17 we have shown that cryoprecipitate corrected completely or partially the BT in three of five patients with severe vWD, all enrolled also in the present study and evaluated with similar criteria. Within the limits of any indirect comparison, it would appear that the effect of cryoprecipitate on the BT is not clearly superior to that of virus-inactivated concentrates.

A surprising finding was that the vWF concentrate, manufactured purposely for the treatment of vWD, was the least effective concentrate, because in no patient was the BT partially or fully corrected. These results are in striking contrast with those of a recent preliminary report of Rothschild et al25 indicating that the concentrate (administered at a dose of 60 U/kg of Ricof) completely corrected for up to 4 hours the BT in three patients with severe vWD. Because the same BT method, the same type of vWD patients, and very similar Ricof doses were used, it is difficult to reconcile the contrasting results of the two studies. There may be lot-to-lot variation in the preparation of this concentrate, which is still experimental; in fact, Mazurier et al30 and Rothschild et al25 state that large molecular weight multimers were present in their batches, whereas we found them missing in our batch.

In an attempt to find variables that could explain the different effects of these concentrates on the BT, we considered Ricof levels and the multimeric structure of vWF. Our goal of attaining levels of Ricof of more than 100 U/dL was achieved in the majority of cases. Hence, the attainment of normal or even supranormal levels of Ricof to FVIII:C endogenous synthesis, so that at 24 hours levels were no lower than those for the remaining concentrates.

**Fig 5.** The same as for Fig 3, but before and after very high purity vWF.
does not secure a normal BT after concentrates. The other variable that we considered was the multimeric structure of vWF. None of the concentrates had an intact multimeric structure, perhaps because larger multimers are lost during the purification process. Loss of larger multimers was accompanied by indirect evidence for heightened proteolytic degradation of vWF, because the more prominent subunits seen in all concentrates, but not in plasma, are usually considered a sign of increased proteolytic cleavage of the constituent subunit of vWF. On the whole, our multimeric analysis indicates that a normal BT can be transiently obtained in some instances even if the multimeric structure in concentrates (and hence in postinfusion patient plasma) is not intact and vWF is partially degraded. On the other hand, it is possible that more consistent and sustained corrections of the BT would be achieved if concentrates had an intact multimeric structure.

Our study was performed using nonbleeding patients and therefore we cannot compare the clinical efficacies of these concentrates. Nevertheless, our results may have clinical implications. It is important that all concentrates did correct the FVIII:C defect, because FVIII:C levels are the main determinant of soft-tissue and postoperative hemorrhages in vWD. For the treatment of these hemorrhages, Hemate P, 8Y, and Alpha VIII should be equally useful. The vWF concentrate should be less useful, particularly during major surgery, because the peak levels, usually aimed at the time of the operation, were actually attained only 6 to 24 hours after the infusion. This situation would require the concentrate to be administered several hours before surgery, which is impractical.

No concentrate consistently normalized the BT in a sustained fashion. This failure might be due to the fact that no concentrate had an intact multimeric structure, but also to the fact that exogenous vWF infused with concentrates does not exchange with platelet vWF, which is usually unmeasurable in severe vWD and plays an important role in securing a normal BT. A normal bleeding time does not seem to be necessary for the control of soft-tissue or postoperative bleeding, whereas it seems to be necessary for the control of mucosal (gastrointestinal, uterine) bleeding. Hence, until a concentrate that consistently and in a sustained fashion normalizes the BT becomes available, some episodes of mucosal bleeding may not be controlled by virus-inactivated concentrates. In these cases, two therapeutic options are available: infusion of DDAVP or platelet concentrates after cryoprecipitate, which in patients with severe vWD have a synergistic effect in normalizing the BT and stopping mucosal bleeding not controlled by cryoprecipitate alone.

REFERENCES

PLASMA CONCENTRATES IN vWD


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