Combined Factor V-VIII Deficiency: A Case Report With Studies of Factor V and VIII Activation by Thrombin

By Mae B. Hultin and M. Elaine Eyster

A new case of combined factor V-VIII deficiency is reported with in vitro studies of factors V and VIII activation by thrombin. The normal activation of factors V and VIII demonstrated in the patient's plasma and the equivalent levels of factor VIII coagulant activity and coagulant antigen support the hypothesis that a quantitative rather than qualitative defect in factors V and VIII is present in this disorder.

APPROXIMATELY 30 cases of combined factor V and VIII deficiency have been reported.1-3 The cause of this rare disorder is not known, but the autosomal pattern of inheritance implies a mechanism distinct from X-chromosome-linked hemophilia A. Whether there is a quantitative or qualitative defect in factors V and VIII is uncertain, but a recent study hypothesized a quantitative defect due to increased destruction of factors V and VIII.3 We report a new case of combined factor V-VIII deficiency with in vitro studies supporting a quantitative rather than qualitative defect.

CASE REPORT

A 32-yr-old white woman was referred to The Milton S. Hershey Medical Center for further evaluation of postoperative bleeding. She was hospitalized for excessive bleeding following tonsillectomy at age 4. Blood transfusions were required for bleeding following the repair of an inguinal hernia at age 25 and 31, and for hemorrhage following cervical polypectomy at age 26 and 32. Dental extractions were accompanied by profuse bleeding, which required packing but no transfusions at age 26. Menstrual periods were normal until age 32 when a dilatation and curettage was performed for dysfunctional uterine bleeding. Fresh frozen plasma, 10 ml/kg, was infused prior to the procedure, which was uncomplicated. Her mother, sister, two brothers, three maternal aunts, three nieces, and four nephews had no history of bleeding. Her father was unknown. Physical examination was unremarkable except for obesity. Her hemoglobin was 14.6 g/dl, her white blood cell count was 8900/cu mm, and her platelet count was 331,000/cu mm.

MATERIALS AND METHODS

The activated partial thromboplastin time (aPTT) and prothrombin time (PT) were performed on the Coag-amate with automated aPTT reagent and Simplastin-automated (General Diagnostics, Morris Plains, N.J.). The thrombin time was performed by a modification of the method of Jim.4 Blood for these routine studies was collected in Vacutainer tubes with citrate (Becton-Dickenson, Rutherford, N.J.). The bleeding time was performed with the Simplate-II device (General Diagnostics).

Blood for all other coagulation studies was collected by the two-syringe technique into plastic tubes containing 1/9 volume balanced citrate (2 volumes 0.1 M citric acid, 2 volumes 0.1 M sodium citrate). Platelet-poor plasma was prepared at 4°C by centrifugation at 9000 rpm for 20 min, and frozen and stored in plastic tubes at −70°C. Reference plasma was obtained from a pool of 10-12 normal donors.

Clot stability for factor XIII deficiency was assessed after a 24-hr incubation of the clot in 5M urea.5 Inhibitors to coagulation factors were sought by measurement of the aPTT manually in mixtures of patient plasma and normal plasma, using Platelein-Plus (General Diagnostics). Mixing studies were also performed with patient plasma and aluminum hydroxide-adsorbed normal plasma or aged normal serum.6 Factors V, VII, and X were measured by the modified one-stage PT assay, using Simplastin and congenital deficient plasma substrate (George King Bio-medical, Overland Park, Kan.), and prothrombin by a two-stage assay.7 Factors VIII, IX, and XI were measured by the modified one-stage aPTT assay as previously described,6 with kaolin and Thrombofax (Ortho Diagnostics, Raritan, N.J.). Factor-VIII-related antigen assays (VIII R:Ag) were performed by the Laurell technique of quantitative electrophoresis, and von Willebrand factor (VIII R:WF) was measured by the modified method of the washed platelet ristocetin assay.8

Studies of the activation of factors V and VIII were performed on aliquots of patient plasma that had been frozen and mailed on solid CO2 to SUNY-Stony Brook and received within 24 hr without evidence of thawing. Subsequent factor V and VIII coagulant (VIII:C) assays of the thawed plasma showed no loss of activity compared to the assays on fresh plasma. The VIII:C assay used to study factor VIII activation was performed as previously reported,9 and factor V activation was assayed by the modified one-stage PT method with congenital factor-V-deficient plasma (George King Biomedical) and Simplastin.9 Fibrometer automatic timers were used for both assays (BBL, Cockeysville, Md.), and the test sample was added last, so that the assay was completed with 1.5 min of subsampling from the activation mixture. Factor V and VIII:C assays were quantitated with a reference curve of pooled normal plasma (PNP) (George King Biomedical).

Patient plasma was diluted 1:1 (v/v) in Tris-buffered saline (0.1 M NaCl, 0.05 M Tris, pH 7.4) (TBS), and a baseline VIII:C assay performed. Purified human thrombin (0.05 U/ml), a gift of Dr. John Fenton, was added to the plasma and serial timed VIII:C assays performed to follow the course of activation and inactivation of VIII:C. A control activation was performed by diluting factor-V-deficient plasma in TBS and factor V-VIII depleted normal plasma (see below) so that the factor VIII level and the total plasma

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concentration was decreased to the same baseline levels (0.08–0.15 U/ml and 50%, respectively) as in the patient plasma study. Thrombin (0.05 U/ml) was then added to the diluted V-deficient plasma, and the activation and inactivation of VIII:C assayed at timed serial points (Fig. 1). The very similar preactivation levels of VIII:C in patient and control studies allowed the VIII:C assays to be performed identically and quickly, by immediate assay of a small subsample without the necessity of a preliminary dilution of the plasma; this assay has a coefficient of variation of ±14%. The activation of factor V in patient plasma and control plasma (factor-VIII-deficient) was studied similarly, with preactivation factor V levels of 0.06–0.10 U/ml (Fig. 2). Artificially depleted factor V-VIII-deficient plasma for these studies was made by incubating fresh oxalated normal plasma at 37°C for 48 hr before aliquoting and freezing at −70°C. The levels of antithrombin III and α2 macroglobulin in patient plasma, congenital V- and VIII-deficient plasmas, and depleted V-VIII-deficient normal plasma, were assayed by radial immunodiffusion (Partigen kits, Calbiochem-Behring, Somerville, N.J.). Factor VIII coagulant antigen (VIII:Cag) was measured in the laboratory of Dr. Leon Hoyer by a method previously reported.¹⁶

RESULTS

Routine coagulation studies revealed a prolonged aPTT and PT, with a normal thrombin time (Table 1). No inhibitor was detected with mixing studies. Correction of the aPTT with equal parts of adsorbed normal plasma but not with equal parts of normal serum in the presence of a prolonged PT suggested an isolated deficiency of factor V or a combined deficiency of factors V and VIII. Specific assays for factors V and VIII were consistently decreased on three occasions. Assays for factors II, VII, IX, X, and XI were normal. Assays for factor-VIII-related antigen and von Willebrand factor were also normal. Factor VIII coagulant antigen was decreased at 17%. No family studies were performed because all family members were either unwilling or unable to cooperate.

![Fig. 1. The activation of factor VIII in plasma. Thrombin (0.05 U/ml) was added to patient plasma (diluted 1:1 with TBS) at "zero" time, after a preactivation VIII:C assay had been performed. Timed serial subsamples were then withdrawn for immediate VIII:C assay (O–O). The preactivation assay was assigned a value of 100% and the subsequent assays expressed in % relative to the preactivation assay. Each point (O) represents the mean ± SEM (denoted by vertical bar) of five independent experiments. A control activation was performed by adding thrombin (0.05 U/ml) to factor-V-deficient plasma with a normal factor VIII:C content, which had been diluted in TBS and factor V-VIII-depleted plasma (1:1 buffer to total plasma volume). Each point is the mean ± SEM of five independent experiments (X–X). There is no significant difference between the two curves at any time point.](image)

![Fig. 2. The activation of factor V in plasma. The activation of factor V in patient plasma (O–O) by added thrombin was compared to that in control plasma (factor-VIII-deficient) (X–X). See legend to Fig. 1 for details. There is no significant difference between the two curves at any time point.](image)

Table 1. Tests of Hemostasis

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Range</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Template bleeding time (min)</td>
<td>&lt; 10</td>
<td>8.5</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>25–45</td>
<td>59</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>9.2–12.8</td>
<td>15.8</td>
</tr>
<tr>
<td>Thrombin time (sec)</td>
<td>18–22</td>
<td>21</td>
</tr>
<tr>
<td>Factor XIII screen</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Inhibitor screen (sec)</td>
<td>49 (1:1 PNP + buffer)</td>
<td>52 (1:1 PNP + patient plasma)</td>
</tr>
<tr>
<td>Factor V (%)</td>
<td>50–150</td>
<td>17, 21, 9</td>
</tr>
<tr>
<td>Factor VIII (%)</td>
<td>50–150</td>
<td>27, 24, 13.5</td>
</tr>
<tr>
<td>Factor II (%)</td>
<td>50–150</td>
<td>102</td>
</tr>
<tr>
<td>Factor VII (%)</td>
<td>50–150</td>
<td>108</td>
</tr>
<tr>
<td>Factor IX (%)</td>
<td>50–150</td>
<td>86</td>
</tr>
<tr>
<td>Factor X (%)</td>
<td>50–150</td>
<td>122</td>
</tr>
<tr>
<td>Factor XI (%)</td>
<td>50–150</td>
<td>108</td>
</tr>
<tr>
<td>Factor VIII R:Ag (%)</td>
<td>50–200</td>
<td>131</td>
</tr>
<tr>
<td>Factor VIII R:WF (%)</td>
<td>40–120</td>
<td>123</td>
</tr>
<tr>
<td>Factor VIII:Cag (%)</td>
<td>55–143</td>
<td>17%</td>
</tr>
</tbody>
</table>
Further studies were performed to test the hypothesis that a qualitative defect might be reflected by diminished thrombin activation of factors V and VIII in patient plasma. The studies are similar in design to the original demonstration by Rapaport et al. that low concentrations of thrombin added to normal plasma markedly shortened its clotting time by activating factors V and VIII. The activation and inactivation of VIII:C in the patient plasma was very similar to that in the control plasma (Fig. 1). The peak of activation of VIII:C in patient plasma (3.6-fold) was 75% of that in the control (4.8-fold), but this difference was not statistically significant. Likewise, the activation and inactivation of factor V in control and patient plasmas were not significantly different (Fig. 2). Both control studies were designed to achieve similar concentrations of factors V, VIII:C, and thrombin inhibitors as in the patient plasma (see Fig. 1 legend) and were based on the assumption that the factor VIII in congenital factor-VIII-deficient plasma, and the factor V in congenital factor-VIII-deficient plasma, were normal. Antithrombin III and α2-macroglobulin concentrations in patient and control plasmas were very similar (<20% difference).

**DISCUSSION**

It is likely that the combined deficiency of factors V and VIII is due to a common defect in some aspect of postsynthetic processing of these proteins, since the structural genes appear to be located on different chromosomes. Marlar and Griffin have recently reported a deficiency of an inhibitor to activated protein C in the plasmas of 4 patients with the combined deficiency. Since activated protein C inactivates factors V and VIII in vitro, they postulated that increased destruction of factors V and VIII by the unopposed action of activated protein C may explain the combined deficiency. On the other hand, Giddings et al.* postulated a qualitative defect common to both proteins based on the detection of normal levels of factors V and VIII antigenic material by inhibitor neutralization assays in some patients, and normal levels of factor-VIII-related antigen by Laurell assay. However, inhibitor neutralization assays provide only semiquantitative estimates of VIII:C Ag, which can now be measured with great accuracy by radioimmunoassay.

We have shown for the first time that VIII:C Ag is decreased in a patient with a combined deficiency of factors V and VIII. In addition, we have demonstrated that thrombin activation and inactivation of factors V and VIII are normal. Our studies were similar in design to the original demonstration of Rapaport et al., who found that thrombin produced a fivefold activation of factor VIII and a threefold activation of factor V when added in comparable amounts to normal plasma. Our studies do not rule out every possible type of qualitative defect. For instance, our patient could have a mixture of normally active and completely inactive molecules. However, this possibility is less likely with the finding that VIII:C Ag and VIII:C were similarly decreased. These studies add support to the hypothesis that a quantitative rather than a qualitative defect is present in patients with a combined deficiency of factors V and VIII.

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