A Procedure for Removing High Titer Antibodies by Extracorporeal Protein-A-Sepharose Adsorption in Hemophilia: Substitution Therapy and Surgery in a Patient With Hemophilia B and Antibodies

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A 37-yr-old hemophiliac with factor IX antibody in high titer required operation for a pseudotumor growing invasively into the bone in his left elbow. Exchange plasmapheresis was not feasible, mainly because of possible stimulation of factor IX inhibitor. In addition, the patient had antibodies against tissue antigens. The factor IX antibodies were of IgG type and adsorbable to protein-A-agarose. The antibodies were removed by extracorporeal adsorption of the plasma (separated intermittently in a cell centrifuge) to sterile protein-A-Sepharose in columns, operated by a computer. The treated plasma was retransfused. In this manner, a total of 6000 ml of plasma was depleted of antibodies, and the antibody titer, as well as the total immunoglobulin content, decreased to one-fifth of the original values. The remaining antibodies were neutralized by infusion of factor IX concentrate. Conventional substitution therapy, in combination with immunosuppression, was then possible. The operation was performed without complications. No signs of hemolysis, complement activation, or activation of the coagulation system occurred. In principle, the same form of treatment can be used in patients with other forms of antibodies.

The most serious complication of hemophilia A and B is the development of antibodies to factor VIII and factor IX. It occurs in 8%-20% of patients with severe hemophilia A and B.1 Transfusions markedly raise the antibody level after 4-5 days. The antibodies have been found to belong to the IgG class of the immunoglobulins.1 Low titer antibodies may be overcome temporarily by large doses of factor VIII or factor IX combined with immunosuppressive agents to delay the secondary immune response to factor VIII or factor IX.3,4 Extensive plasmapheresis and massive amounts of factor VIII have occasionally been tried in patients with high titer antibodies.5-7 In another approach, activated factor IX concentrates have been used to achieve hemostasis in patients with antibodies against factor VIII.8-12 Such treatment has, as far as we know, never been used in patients with hemophilia B and antibodies. In hemophilia B with antibodies in high titer, the situation is still worse because all commercially available factor IX concentrates contain small amounts of activated coagulation products,13-15 capable of leading to intravascular coagulation when used in large doses.

This report concerns a patient with severe hemophilia B and antibodies to factor IX in high titer. After removal of antibodies to factor IX by extracorporeal adsorption of the plasma with Fc-reactive protein-A of Staphylococcus aureus covalently linked to agarose (Protein-A-Sepharose CL-4B), the patient could be treated with conventional replacement therapy so that orthopedic surgery could be carried out.

CASE REPORT

The patient, aged 37, was a severely affected hemophiliac who had had repeated severe bleeding episodes during childhood and had received about 300 blood transfusions at his local hospital. When first seen at the Coagulation Laboratory in Malmö at the age of 15, he was found to have an inhibitor in high titer against factor IX (the inhibitor was demonstrable in dilution 1/500 of the plasma). From then on, blood products were given only occasionally. After these infusions the inhibitor level rose 20-50-fold within a few days. During the last 8 yr he had not received any infusions.

The patient has several joint defects. In the autumn of 1979 he developed a pseudotumor in the left elbow. The tumor grew quickly. Computed tomography showed that the pseudotumor had caused wide destruction of the cortex of the proximal ulna and adjacent cystic changes extending to the radius. Because of an immense risk of perforation and fracture, surgery was considered necessary.

In the last few years the antibody level, assayed according to the method of Nilsson and Hedner,7 has fluctuated between 6 and 7 U/ml corresponding to about 20 Bethesda units of inhibitor/ml.16 The inhibitor was quantitatively adsorbed to a column of protein-A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) and quantitatively eluted from such a column with 0.1 M glycine-HCl buffer, pH 2.4.

The patient had antibodies to Kell antigen and leukocyte agglutinins. His blood status was otherwise normal. He weighed 48 kg and his plasma volume was 2315 ml.

MATERIALS AND METHODS

Blood Collection

Citrated platelet-poor plasma was prepared as described previously.17,18 Plasma samples were tested immediately or stored at −70°C until used.

As reference for the coagulation assays we used pooled citrated plasma from 20 normal individuals.
Coagulation Tests

Platelet count, factor VIII coagulant activity (VIII:C), factor VIII related antigen (VIII:R:Ag), P&P (prothrombin plus factor VII plus factor X), factor V, fibrinogen, fibrinogen/fibrin degradation products (FDP), and ethanol gelation test were determined or performed according to methods described elsewhere.\(^a\)\(^b\)\(^c\)\(^d\)\(^e\)\(^f\)\(^g\)\(^h\)\(^i\)\(^j\)\(^k\)\(^l\)\(^m\)\(^n\)\(^o\)\(^p\)\(^q\)\(^r\)\(^s\)\(^t\)\(^u\)\(^v\)\(^w\)\(^x\)\(^y\)\(^z\)

Factor IX

Factor IX clotting activity (IX:C) was assayed with a one-stage assay\(^1\)\(^2\)\(^3\)\(^4\) and factor IX clotting antigen (IX:CAg) with a solid-phase immunoradiometric method based on homologous antibodies from a hemophilic patient.\(^5\) The reference plasma was said to contain 1 U/ml plasma. The results were expressed in U/ml.

Quantitative Determination of Factor IX Inhibitor\(^4\)

Various dilutions of the plasma to be tested (0.6 ml) were incubated with 0.2 ml of a factor IX concentrate (Preconativ, KabiVitrum, Sweden) containing 3 U factor IX/ml, at 37°C for 15 min. As a blank 0.6 ml hemophilia B plasma (less than 1 U/dl IX:C and no inhibitor) was incubated with 0.2 ml of the factor IX concentrate. Following incubation, the mixtures were assayed for residual IX:C in the way described above. The inhibitory activity of the plasma was expressed as the number of units of IX:C (1 U of IX:C is defined as the amount of IX:C present in 1 ml normal plasma) inactivated by 1 ml of the plasma.

Factor IX has also been estimated according to Miller-Andersson et al.\(^3\)\(^2\) One bottle (20 ml) contained 500 U IX:C and 3 U bin III (AT III) was determined both by electroimmunoassay\(^2\)\(^4\) and by amidolytic method (S2238).\(^2\)\(^5\)

Immunochemical Determination of IgG

Quantitation of IgG, IgA, and IgM was performed by an immunochemical procedure.\(^2\)\(^7\)

Assay of Complement Factors

The concentrations of the following complement components, C3, C4, Clq, and Clr, were estimated by electroimmunoassay according to Sjöholm.\(^2\)\(^8\) Leukocyte agglutinins were estimated according to Kissmeyer-Nielsen.\(^2\)\(^9\)

Ionized calcium was determined by a calcium ion selective electrode and a double junction reference electrode using the flow injection analysis technique.

Factor IX Concentrate

Preconativ (KabiVitrum, Sweden) was prepared according to a slight modification of the method of Tullis et al.\(^2\)\(^0\) It was prepared from platelet-poor plasma, but without addition of heparin during the procedure.\(^2\)\(^1\) One bottle (20 ml) contained 500 U IX:C and about 1000 U IX:CAg.

Antithrombin III Concentrate

This was obtained from KabiVitrum. It was prepared by the method described by Miller-Andersson et al.\(^2\)\(^2\) One bottle (20 ml) contained 500 U AT III.

Equipment for Serial Plasmapheresis and Removal of Antibody from Patient Plasma

Patient monitor. To obtain an even balance on the outgoing and ingoing fluid volume, a pumping monitor with a safety device and an electronic bedside were used. The pumping device consisted of two blood pumps and a pump for the anticoagulant (0.13 M sodium citrate). The safety devices of the monitor were an ultrasonic air detector, a patient pressure gauge, and a solenoid valve (BMM10/ITM, Gambro AB, Lund, Sweden).

Plasma separator. The patient monitor delivered whole blood at a suitable flow rate to a reservoir connected to the Haemonetics Model 30 Cell Separator (Haemonetics Inc., Natick, Mass.). This was used according to the operation manual, except that the citrate pump was bypassed. Separated plasma was collected in sterile sealed bags and transferred to the plasma treatment monitor. The erythrocyte concentrate was pumped up to another reservoir where it was mixed with treated plasma and retransfused to the patient via the patient monitor.

Plasma treatment monitor (CITEMS-1A). For sterile handling of the plasma, a special monitor was built consisting of three parts: (1) a modular monitor with pumps, solenoid valves, and safety devices; (2) analysis devices for process control and chart recording; (3) computer for process control including printer registration. The modular monitor had two pumps, one for the plasma and one for the solutions for elution, and valves for choice of fluid during the elution phase. A drip chamber with an ultrasonic air detector was placed upstream from the columns as well as a pressure transducer with an alarm. Downstream from the columns similar valves were used for separating treated fluids into plasma and elution fractions. Analysis devices for pH and UV were placed in each line to detect pH changes and plasma protein concentrations. All parameters were recorded and printed continuously. The elution monitor could simultaneously pass the plasma to be treated through one column and elute the other. Elution was made by gradually lowering of pH. The elution period was divided into five phases: (1) saline rinsing of residual plasma; (2) pH gradient elution 7.2–2.5; (3) flushing at low pH; (4) restoring to normal pH; (5) saline flushing. Thereafter, the column was ready for a new portion of plasma. Programs for process control were made by the project group and its consultants at Gambro Research AB, Lund, Sweden.

Columns. The system included two 147-mI columns for gel chromatography (Gambro AB) containing sterile and pyrogen-free protein-A-Sepharose CL-4B (Pharmacia Fine Chemicals AB, Uppsala, Sweden). The housing of the columns was sterilized with ethylene oxide as sterile disposable products, approved by FDA, and thereafter they were packed with the gel. The purity of the columns was finally checked by bacteriologic and pyrogen tests. The binding capacity is approximately 3 g of IgG/column. The actual highest flow rate used was 15 ml/min. To avoid hyperhydration of the patient, no saline-diluted plasma was retransfused. This volume corresponds to an estimated loss of plasma of about 3%.

PROCEDURE AND RESULTS

On two consecutive days, starting on the day before the day planned for the operation, the patient’s plasma was depleted from antibodies by the procedure described below. Plasma from the patient was obtained by using the Haemonetic Cell Separator (Fig. 1). The arterial blood flow from the patient and the inflow of treated plasma mixed with earlier separated erythrocytes into the patient were controlled by calibrated blood pumps to secure hemodynamic balance. Sodium citrate, 0.13 M, at the ratio of 1:19, was used as anticoagulant. The patient himself was not heparinized. The adsorption was performed continuously but, as a precautionary measure, the
plasma received was transferred in portions to the fully automated apparatus for elimination of the immunoglobulins. On the first day of treatment, 9 portions (total 3600 ml) of plasma were treated within a period of 6 hr. The levels of factor IX antibodies in the different portions are given in Table 1. The next morning, about 12 hr after completed treatment, the antibody level had increased from 1.6 to 2.3 U/ml plasma and the IgG values from 4 to 5 g/liter. The same procedure was then repeated and 2400 ml of plasma was reinfused after elimination of the immunoglobulins, including factor IX antibody. At the end of the second period of treatment the antibody titer reached the level of 1.2 U/ml plasma and IgG 2.4 g/liter. The patient tolerated the treatment very well.

During, between, and after the two periods of adsorption of plasma, a battery of tests was performed including platelet counts, assays of fibrinogen, IgG, fibrin monomers (ethanol gelation test), FDP, P&P, VIII:C, VIIIIR:Ag, IX:C, IX:CAg, V, antithrombin III, ionized calcium, C3, C4, Clq, C1 (Table 2). Except for a slight reduction of the platelet count, no pathologic changes were observed.

Conventional treatment with factor IX was then possible (Fig. 2). In order to neutralize the residual antibodies and to raise the level of factor IX to about 150 U/dl, 7500 U of factor IX were needed, and 2500 U of antithrombin III was also given to prevent activation of the coagulation system. When the loading dose of factor IX was given, the patient developed an anaphylactic reaction with edema and urticaria after infusion of the approximate dose calculated necessary to neutralize the remaining inhibitor. He was treated with calcium and an antihistaminic drug in accordance with the local routine practice. Further doses of factor IX did not produce any reactions. To suppress antibody synthesis, which was expected to accelerate because of withdrawal of antibody, cyclophosphamide was given from the first day of plasmapheresis in a dose of 500 mg i.v. for 2 days and then 50 mg p.o.
Fig. 2. The course of treatment.

Table 2. Results of Assays Performed Before, During, and After the Start of the Plasmapheresis Procedure

<table>
<thead>
<tr>
<th>Samples Obtained</th>
<th>Normal Area</th>
<th>3 Days Before Plasmapheresis</th>
<th>Relative to First period of Plasmapheresis</th>
<th>Relative to Second Period of Plasmapheresis</th>
<th>After Treatment With F IX</th>
<th>3 Days After Plasmapheresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-Hemoglobin (g/liter)</td>
<td>131–163</td>
<td>98</td>
<td>119</td>
<td>99</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>B-Haptoglobin (g/liter)</td>
<td>0.3–1.8</td>
<td>1.1</td>
<td>1.8</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-Platelets (x 10^9/liter)</td>
<td>125–340</td>
<td>151</td>
<td>176</td>
<td>125</td>
<td>110</td>
<td>106</td>
</tr>
<tr>
<td>B -Platelets (x 10^9/liter)</td>
<td></td>
<td></td>
<td>250</td>
<td>95</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>P-Ionized calcium (ppm Ca^{2+})</td>
<td>&gt; 100</td>
<td>95</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>~ 100</td>
<td>~ 100</td>
</tr>
<tr>
<td>P-Fibrinogen (g/liter)</td>
<td>2.0–4.0</td>
<td>5.1</td>
<td>3.9</td>
<td>3.6</td>
<td>3.7</td>
<td>3.6</td>
</tr>
<tr>
<td>P-IgG (g/liter)</td>
<td>7–15</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>P-C4 (% of a normal standard)</td>
<td>53–207</td>
<td>165</td>
<td>197</td>
<td>215</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B, blood; P, plasma.

times/day for 8 days (Fig. 2). The patient was operated on with removal of the blood-filled cyst. During the latter part of the operation, the patient was given another 1500 U of factor IX because of a reduction of the factor content from 155 to 90 U/dl. A level of factor IX of 90–145 U/dl was maintained during the next 2 days by administration of another 1500 U every 4 hr, corresponding to 8 U/kg body weight/hr. The maintenance dose was thereafter reduced to 1500 U every 8 hr. The factor IX level was thus kept at about 100 U/dl for 6 days. Simultaneous determinations of IX:Cag showed substantially higher values in the range of 180–270 U/dl.

No further antithrombin III was given until the third day after the operation. The patient then showed signs of activation of the coagulation and fibrinolytic systems with positive ethanol gelation test and FDP present in the blood. Treatment with 1500 U of AT III
at each time of administration of factor IX concentrate was started, and the coagulation status became normal.

Seven days after the operation, the antibody was again demonstrable in plasma. In an endeavor to delay the antibody rise, another dose of 5000 U of factor IX was given 3 times in the course of 16 hr as well as 2 mg of vincristine, but without any demonstrable effect. As expected, during the postoperative course, the antibody titer rose to a maximum of 50 U/ml plasma on the 16th day after the operation. The titer gradually decreased, but 7 mo later it still was about 15 U/ml plasma. The lowest IgG level recorded was 2.4 g/liter, but within 3 days it was 6. The patient received antibiotic therapy, and he did not show any signs of infection. No abnormal bleeding occurred during or after the operation, and the wound healed without delay.

**DISCUSSION**

In this case of severe hemophilia B and antibody against factor IX it was possible to prepare the patient for substitution therapy and orthopedic surgery by serial runs with extracorporeal adsorption of the immunoglobulins, including the factor IX antibody to protein-A-Sepharose, and retransfusion of this antibody-depleted plasma.

Hemophiliacs with high titers of antibodies against factor IX have hitherto been regarded as virtually resistant to therapy. To neutralize the antibody in the intra- and extravascular space and to raise the factor IX level to about 80 U/dl, this patient would have required about 700 U of factor IX/kg body weight. The largest dose we have administered on a single occasion is 150–300 U/kg body weight, and then signs of intravascular coagulation appeared. At first we considered the possibility of serial plasmapheresis with fresh-frozen normal plasma as replacement fluid to lower the antibody level. Such treatment was, however, considered too hazardous because of expected side effects of such replacement fluid owing to the patient’s leukocyte agglutinins. In addition, the factor IX of such an infusion would hasten the secondary immune response.33 Serial plasmapheresis with the use of saline and albumin as replacement fluid was also considered. Such exchange transfusions are reportedly followed by a significant fall in platelet count, fibrinogen level, and other coagulation factors.34 35 Introduction of multiple coagulation defects in a severe hemophilic implies a substantial increase of the risk of bleeding. Exchange transfusion was therefore considered contraindicated in this case.

The antibodies to factor IX in the present case were adsorbable to protein-A-Sepharose. This protein does not adsorb IgG36 Thus, the factor IX antibody must consist mostly of other IgG subclasses. Extracorporeal immunoglobulin adsorption by killed protein A containing staphylococci37 and by protein-A-Sepharose (Jonsson and Håkansson, personal communication, 1980) has been found to be a safe procedure in patients with malignant tumors. We therefore thought that the best procedure for lowering the antibody level in the patient would be serial adsorption of the patient’s plasma with protein-A-Sepharose. One possible risk is leakage of protein-A-Sepharose, but the columns had been checked, and no leakage whatsoever had been discovered. Our case, in contrast to the malignant cases on record, required adsorption of more than 90% of the immunoglobulins from about 6 liters of plasma. The apparatus described above met this requirement. Thus, by passing the patient’s plasma through columns of protein-A-Sepharose, it was possible to adsorb the specific antibodies together with the bulk of immunoglobulins. After retransfusion of 3600 ml of adsorbed plasma on the first day of treatment, the patient’s antibody level fell from 6 to 2.4 U/ml and his IgG level from 10 to 4 g/liter. It has been reported that, after exchange transfusions, the antibody level rises rapidly because of diffusion of antibodies from the extravascular compartment and because of stimulation of antibody synthesis.24 26 38 Cobcroft et al.7 also pointed out that serial plasmapheresis should be delayed until the peak antibody level has been passed. The second series of plasmapheresis in our case was performed after an interval of about 12 hr. During this period, the antibody level had risen only slightly, which indicates that with this procedure the antibodies in the intra- and extravascular compartments became virtually equilibrated within the 6 hr that the first series of plasmapheresis required. The fact that the cyclophosphamide was given from the start might have helped to suppress synthesis of antibody. After completion of the second series of plasmapheresis (we had then adsorbed and retransfused 6 liters of plasma), the antibody level had fallen to one-fifth of its original level, and substitution therapy was possible. It is remarkable that all these manipulations of the blood and the plasma were not followed by any side effects. The patient did not show any signs of hemolysis. Nor were any signs of consumption of complement components observed. There was only a slight fall in the platelet count, while the values for the various coagulation factors remained normal. Marked reduction in ionized calcium has been observed not only in normal blood donors undergoing plasmapheresis, but also in patients receiving rapid infusions of blood anticoagulated with
citrate. The patient was followed with repeated measurement of ionized calcium but no reduction was observed.

As for the substitution therapy, that customarily used in the treatment of patients with low inhibitor titer was given. The patient was thus given cyclophosphamide combined with factor IX concentrates. Simultaneous determinations were made of IX:C and of IX:CAG. In comparison, the antigen level was substantially enhanced, indicating that the preparations contained inactive factor IX. In this case, AT III was added from the beginning. However, as no side effects of the basic treatment were observed, AT III was withdrawn. When signs of activation of the coagulation system appeared on the third day after the operation, AT III was again added to the treatment, and the laboratory test results were normalized within a day. It seems reasonable to use this drug to counteract activation of the coagulation process introduced by factor IX concentrates. The effect of the immunosuppressive treatment is of course difficult to evaluate in this case. In view of an earlier report, we judged that renewed synthesis of antibodies could be suppressed for 7 days in this case. Later treatment with vincristine after the antibodies had reappeared had no demonstrable effect.

Thus, treatment with extracorporeal protein-A-Sepharose chromatography made customary substitution therapy possible in our patient with hemophilia B and antibodies by lowering the originally high antibody titer. This system should permit removal of an antibody with a 2–4 times higher titer corresponding to 40–80 Bethesda units. Consequently, it seems possible to use the same technique in other cases with antibodies, for instance against factor VIII and platelet antigens. As pointed out, we could not suppress the secondary antibody response, and now the problem is what treatment can be offered immunized hemophiliacs in clinical situations where maintenance of normal clotting activities is required for several weeks. Our next tasks are, therefore, first, to try to remove antibodies by specific immunoadsorption and, second, to improve the pharmacologic immunosuppressive treatment.

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REFERENCES

22. Hedner U, Nilsson IM: Parallel determinations of FDP and
A procedure for removing high titer antibodies by extracorporeal protein-A-sepharose adsorption in hemophilia: substitution therapy and surgery in a patient with hemophilia B and antibodies

IM Nilsson, S Jonsson, SB Sundqvist, A Ahlberg and SE Bergentz