Failure of Combined Factor VIII and Cyclophosphamide to Suppress Antibody to Factor VIII in Hemophilia

By Marilyn A. Hruby and Irving Schulman

Two patients with hemophilia A and anti-factor VIII antibodies were treated with infusions of factor VIII concentrates and intravenous cyclophosphamide in an attempt to suppress the antibody response. Factor VIII levels of 23% and 95% were achieved immediately postinfusion, and prompt control of bleeding ensued. Sequential antibody titers demonstrated no change in antibody response in either patient when compared to previous studies following factor VIII infusion alone. These results are in contrast to the previously reported suppression of factor VIII antibody in a nonhemophilic patient using an identical regimen.

The development of anti-factor VIII antibodies in a hemophilic patient represents a major complication in the control of hemorrhage. Recent surveys of large groups of hemophilic patients indicate the incidence of such antibodies may be as high as 20%. Various types of immunosuppressive agents including corticosteroids, 6-mercaptopurine, and azathioprine have been used without success in an effort to modify the rise in antibody titer following factor VIII infusion.

Green recently reported suppression of acquired factor VIII antibody in a nonhemophilic patient with the combined use of a large dose of factor VIII concentrate followed immediately by intravenous cyclophosphamide. We undertook to evaluate this regimen in two hemophilic boys, both of whom had had previous sequential inhibitor titers measured following infusion of factor VIII alone.

METHODS AND MATERIALS

Two unrelated patients with severe factor VIII deficient hemophilia were studied. B.J. is a 5-yr old boy who was found to have hemophilia at 2 days of age and in whom factor VIII antibody was first detected at age 18 mo. At the time of this study he required factor VIII therapy for prolonged bleeding from a small tongue laceration. S.J. is a 17-yr-old boy first diagnosed as a hemophilic at 6 mo of age and who developed a factor VIII antibody at 11 yr of age. Prolonged bleeding after dental extraction prompted therapy with factor VIII at the time we began these observations. Patient B.J. weighed 15 kg, and patient S.J. weighed 45 kg at the time of treatment.

Both patients received Hyland AHG concentrate (Hyland Div. Travenol Laboratories, Inc., Costa Mesta, Calif. 92626) 200 U/kg as a rapid infusion followed immediately by intravenous cyclophosphamide (Cytoxan) 25 mg/kg at a time when their antibody titers were less than 0.5 U.
Prompt cessation of bleeding ensued in each patient. Sequential inhibitor titers were measured frequently in both boys and the response pattern compared to their previous response to factor VIII infusion alone.

Venous blood for coagulation studies was collected through 21-gauge scalp vein needles into plastic syringes and transferred directly to plastic tubes containing buffered citrated anticoagulant in a ratio of nine parts of blood to one part of anticoagulant. Plasma was prepared by centrifuging citrated blood at 10,000 rpm for 15 min at 4°C.

Activated partial thromboplastin times (normal range 30–45 sec) and factor VIII assays (normal range 50–150%) were performed according to the method of Simone et al.6

Factor VIII inhibitor titers were measured by first preparing twofold dilutions of the patient’s plasma using a diluting fluid (1 part 0.1 M sodium citrate and 6 parts of 0.9%, sodium chloride). One-tenth cubic centimeter of each dilution was then incubated with 0.1 cc pooled normal plasma for 30 min at 37°C. A modified partial thromboplastin time was performed according to the method of Simone et al.6 using 0.1 cc of each incubated mixture. The clotting times in seconds were plotted against the dilution of inhibitor plasma. The inhibitor titer was taken as the reciprocal of the lowest dilution of patient’s plasma that completely neutralized the factor VIII present in normal plasma (Fig. 1). Therefore, 1 U of inhibitor represents the amount of inhibitor that would neutralize the factor VIII present in 1 ml of average normal plasma.

**RESULTS**

Postinfusion factor VIII levels in patient B.J. were 95% at 20 min, 39% at 4 hr, and less than 1% at 24 hr. In patient S.J. the factor VIII level was 23% at 20 min postinfusion and less than 1% at 6 hr. Pretreatment factor VIII antibody titers were less than 0.5 U in both patients.

Figure 2 compares the inhibitor response in patient B.J. after infusion of factor VIII alone from a previous study and of factor VIII followed by cyclophosphamide. Figure 3 compares the inhibitor response in patient S.J. under
Fig. 2. Response of factor VIII antibody titer in patient B.J. following factor VIII infusion alone (broken line) and factor VIII followed by cyclophosphamide (solid line).

Fig. 3. Response of factor VIII antibody titer in patient S.J. following factor VIII infusion alone (broken line) and factor VIII followed by cyclophosphamide (solid line).
similar conditions. No modification of inhibitor response was achieved in either patient using cyclophosphamide in this manner.

DISCUSSION

Previous studies in hemophilic boys with anti-factor VIII antibody have demonstrated a characteristic pattern of antibody response following factor VIII infusion. This typical response includes a lag phase of 5–7 days when little or no antibody can be detected, followed by a rapid rise in antibody titer reaching a peak in 2–3 wk, followed by a gradual decay over months or years. Efforts to modify or suppress this antibody response using immunosuppressive drugs have been disappointing.

Green reported the disappearance of a long-standing acquired anti-factor VIII antibody in a nonhemophilic patient by the use of a large dose of factor VIII followed by infusion of cyclophosphamide. This regimen was based on the hypothesis that (1) significant levels of free factor VIII would result in stimulation of antibody-producing cells, and (2) these susceptible cells would be destroyed by the cytotoxic agent. Our hemophilic patients were treated by an identical regimen using a large dose of factor VIII concentrate which resulted in an immediate free factor VIII level of 95% in patient 1 and 23% in patient 2. Nevertheless, in contrast to the previous report, no change in the characteristics of the antibody response could be demonstrated. One explanation for this difference in response of nonhemophilic and hemophilic patients may be that the pathogenic mechanism resulting in factor VIII antibodies is different in the two groups. Other reports of spontaneous or possible drug-induced remissions of anti-factor VIII antibodies in nonhemophilic patients would tend to support this idea.

Since the present study was begun, other reports have appeared which claim some benefit was achieved by similar regimens of combined cyclophosphamide and specific factor replacement in hemophiliacs with antibodies. Lusher and Evans treated two factor VIII-deficient hemophiliacs with intravenous cyclophosphamide 10 mg/kg, followed immediately by factor VIII concentrate, and an additional 3-day course of oral cyclophosphamide. No subsequent rise in antibody titer was noted. However, measurable factor VIII levels were not achieved in these patients, and one may question whether sufficient antigen was present to stimulate antibody production. Nilsson et al. reported temporary suppression of anti-factor IX antibody production in two factor IX-deficient hemophiliacs using combined cyclophosphamide and factor IX concentrates followed by a 10-day course of oral cyclophosphamide. The authors noted that a delay in reappearance of the antibody for as long as 3 mo could be achieved by this regimen, providing factor IX doses were large enough to yield a level of 50% or more and neutralize the antibody. In a third report, Edson et al. described a hemophilic patient with anti-factor VIII antibody who received exchange transfusion, and treatment with corticosteroids, large doses of factor VIII concentrates, and several doses of cyclophosphamide over a 1-mo period. It was concluded by the authors that immunosuppression was effective in reducing antibody production, although the anamnestic response was not
delayed. Obviously the effect of cyclophosphamide is more difficult to assess
in such a complicated situation.

The major difference in therapeutic approach between the present report and
those of Lusher and Evans and Nilsson et al. seems to be the addition of oral
cyclophosphamide for 3-10 days following the simultaneous infusion of cyclo-
phosphamide and specific factor concentrates. Based on our results it would
seem appropriate to suggest that the further use of a single intravenous dose of
cyclophosphamide as employed by Green and ourselves is not warranted in
hemophilic patients with antibodies, and that further trials to assess the effect
of cyclophosphamide should, in addition, utilize a more protracted course of
oral therapy. Adequate evaluation of the benefit of such therapy can be made
only if the prior established pattern of antibody response in each patient is
known and if a uniform method of measuring antibody titers is established
to facilitate comparison of data from different centers.

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