Effect of Danazol on Clotting Factor Levels, Bleeding Incidence, Factor Infusion Requirements, and Immune Parameters in Hemophilia

By Parvin Saidi, Beatriz Z. Lega, Hugh C. Kim, and Karel Raska, Jr

Several recent studies have reported conflicting results on the effectiveness of danazol, an attenuated androgen, in raising plasma levels of clotting factors VIII and IX in patients with hemophilia. We undertook a randomized, double-blind cross-over trial using 8 weeks' administration of danazol (D), 600 mg/d, and 8 weeks' administration of placebo (P) separated by 2 weeks of rest in 12 patients with hemophilia A and four patients with hemophilia B. Plasma factor VIII and IX levels, frequency and type of bleeding episodes, amount of factor concentrate infused, fibrinogen, fibrinolysis assays, antithrombin III, liver function, and immune parameters were followed. During the danazol phase a minimal increase was noted in the average clotting factor levels, an increase that, although statistically significant, was of hemostatically marginal magnitude. Significant increases in protein C and plasminogen levels, however, were observed during the danazol period, suggestive of danazol-mediated enhanced fibrinolysis. Clinically, bleeding frequency was significantly increased, and more clotting factor was consumed during the danazol period. Furthermore, eight episodes of hematuria and oral mucosal bleeding was reported during the danazol phase in contrast to only one episode of hematuria during the placebo phase, consistent with an enhancement of fibrinolytic activity with danazol. We conclude that danazol does not have a hemostatically significant effect on plasma levels of factor VIII and IX but may be associated with enhancement of fibrinolytic activity, resulting in increased bleeding frequency and requiring more clotting factor infusions. Therefore, danazol is not a viable alternative in the treatment of hemophilia.

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The availability of clotting factor concentrates has greatly facilitated replacement therapy for the acute bleeding episodes in hemophilia and has vastly improved the general health and lifestyle of this patient population. There has, however, been a growing concern regarding the potential long-term complications associated with chronic and/or excessive use of such factor concentrates. High incidence of acute hepatitis, chronic liver disease,1 and more recently human T-cell lymphotropic virus Type III (HTLV-III)-positive antibody tests2,3 are well recognized in patients receiving factor concentrates. Additionally, altered immune status, specifically in the number and function of T lymphocyte subsets, has been reported in hemophiliacs exposed to factor concentrates, suggesting a potential predisposition to the acquired immunodeficiency syndrome (AIDS).4,5 Therefore, the availability of agents not derived from blood products but capable of increasing plasma levels of clotting factors VIII and IX would be of obvious advantage. Gralnick and Rick reported in 1983 that danazol, an attenuated androgen, administered at doses of 600 mg/d for 14 days to four patients with hemophilia A and one patient with hemophilia B resulted in a significant increase in their plasma clotting factor levels. However, subsequent studies by others did not consistently confirm these findings.6-13 To further clarify the effect of danazol in a larger number of patients and for a longer duration of treatment, we undertook a double-blind, cross-over study in 22 hemophiliacs treated with danazol or placebo for a period of 8 weeks. Frequency of bleeding episodes, factor concentrate utilization, coagulation and fibrinolysis parameters, and liver function tests were closely followed during danazol and placebo treatment periods. Additionally, since it has been suggested that the action of danazol may, in part, be mediated by an immune mechanism,14 we followed the immune parameters of our patients during this study.

MATERIALS AND METHODS

Patient population. Twenty-two patients initially consented and entered the study, and 16 patients completed the entire protocol. Five patients were excluded from the study within the initial 4 weeks because of noncompliance to protocol, and one patient was removed from the study because of medical problems unrelated to the protocol. All patients participating in this study were advised of procedures and attendant risks, in accordance with institutional guidelines, and gave informed consent.

Sixteen patients completed the entire protocol. Twelve had hemophilia A, six with severe factor VIII deficiency (<1%), three with moderate factor VIII deficiency (1% to 5%), and three with mild factor VIII deficiency (5% to 10%); four patients had hemophilia B, including one with severe factor IX deficiency (<1%) and three with moderate factor IX deficiency (1% to 5%). All patients were older than 18 years of age (mean age, 32 years) and averaged more than six bleeding episodes per year requiring factor infusions. No patient had clinical history or evidence of acute hepatitis within 6 months preceding the study, and no patient had increased levels of inhibitor against clotting factor greater than 1 Bethesda U/mL at any time during the study.

We used a randomized, double-blind cross-over design in which each patient served as his own control. The order in which the drug/placebo was administered was randomly determined. The identity of the drug, which was distributed by the hospital pharmacy, was indicated only by a code number and was known only to the pharmacist. The patients were assigned to start receiving either danazol, 200 mg three times a day, or an identical placebo for a period of 8 weeks; this was followed by 2 weeks of rest, and then they crossed over to the alternate agent. At the end of each phase of the study, a tablet count was done to ensure compliance. Patients kept records of the site and severity of each bleeding episode and the amount of factor concentrate infused. Each patient also completed a questionnaire on the side effects of the treatment at 2, 4, and 8 weeks.

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of the treatment period. Coagulation, euglobulin lysis time, and liver function parameters were obtained 1 month prior to the start of the study, days 0 and 3, then weeks 2, 4, and 8 of each treatment period. Protein C, plasminogen, plasminogen activator, and antithrombin III levels were determined at the end of each treatment period. Immune globulin quantitation, complement levels and lymphocyte numbers, and profile and function were determined at the beginning of the study, day 0, and at the end of each treatment period. Skin testing was done prior to the study and at the end of each treatment period.

Laboratory techniques. Blood was drawn through a 19-gauge butterfly from the forearm and placed in polyethylene tubes containing 3.8% sodium citrate (9 vol of blood to 1 vol of anticoagulant). The plasma was immediately separated, rapidly frozen, and stored at −70 °C. The plasma samples were assayed for prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, specific factor assays (factor VIII or factor IX), euglobulin lysis time (ELT), plasminogen, tissue plasminogen activator (t-PA), and protein C at the completion of the study. For tests of coagulation, all samples from each patient were assayed in one batch using the same lots of reagents (deficient plasma, standard reference plasma, and normal pooled plasma) to eliminate the difference inherent in interassays.

Complete blood counts were done by the use of the Coulter Counter (Coulter Electronics, Hialeah, Fla). Liver function tests, including SGOT, SGPT, GGT (gamma-glutamyl transpeptidase), and alkaline phosphatase were performed in accordance with standard laboratory procedures on the same day that blood samples were obtained.

PT and APTT were performed by the one-stage assay. Fibrinogen levels were assayed based on the thrombin time using Data-Fi Fibrinogen Calibration Reference normal plasma (Dade Diagnostics, Miami). We used the one-stage APTT-based assay for factor VIII and IX with deficient substrate plasma (General Diagnostics, Morris Plains, NJ) and reference normal plasma (Helena Laboratories, Beaumont, Tex). Using three-cycle semilog paper, factor VIII and IX levels were measured as low as 0.5%.

ELT determination was performed with Data-Fi Euglobulin Lysis Reagents for qualitative fibrinolysis testing, and results were expressed as units of activity, with the reciprocal of lysis time of 300 minutes defined as one unit. Plasminogen was determined by the chromogenic substrate method using Spectrozyme PL (American Diagnostica, Greenwich, Conn). Antigenic levels of tissue plasminogen activator was determined by the enzyme-linked immunosorbent assay ELISA technique using goat antiserum against human uterine tissue plasminogen activator (American Diagnostica). Protein C was measured by Laurell’s rocket immuno electrophoresis technique using goat antihuman protein C (American Diagnostica). Antithrombin III was measured by radioimmuno diffusion using rabbit antihuman antithrombin III (Behring Diagnostics, La Jolla, Calif).

Peripheral blood mononuclear cells were prepared from heparinized samples by the standard Ficoll-Hypaque gradient. The T lymphocyte populations were quantitated under standard conditions using Ortho-Immune monoclonal antibodies (Ortho Diagnostics, Raritan, NJ). The B cells were quantitated by staining surface immunoglobulins; the natural killer cells were stained with the Leu-7 monoclonal antibody (Becton Dickinson, Sunnyvale, Calif). The immunofluorescence was estimated by flow cytometry in an Ortho System-50 Cytofluorograf.

Mixed lymphocyte reactions (MLR) were carried in round-bottomed microtiter plates using 7.5 × 10⁶ responder and 7.5 × 10⁶ mitomycin C-treated (30 g/mL for 30 minutes) stimulator cells in RPMI 1640 medium supplemented with 10% pooled human serum as described previously. After an 18-hour pulse with ³H-thymidine on the fifth day, the cells were harvested and processed using the automated cell harvester. Phytohemagglutinin (PHA) stimulation was determined with optimal lectin concentrations and 7.5 × 10⁶ mononuclear cells under the same conditions. The level of IgA, IgG, IgM, C3, and C4 were determined in plasma samples kept at −70 °C by radioimmun assay using Meloy plates (Meloy Laboratories, Springfield, Calif). Skin tests for delayed sensitivity were performed using three recall antigens; PPD, mumps, and Candida. For statistical analysis, the Wilcoxon signed rank test for paired samples (two tailed) was used for comparison between the placebo and danazol phases.

RESULTS

The effects of danazol on clotting factor levels, bleeding frequency, and amount of clotting factor concentrate consumption is shown in Table 1. Of 16 patients evaluated, 12 had hemophilia A, and four had hemophilia B. As a group, the mean factor level during the danazol phase was minimally increased as compared with that of the placebo phase (2.5% ± 1.88% during the danazol phase vs 2.0% ± 1.96% during the placebo phase,  P = .0002). Although the incremental increase of mean factor levels for the patients as a group during the danazol phase was statistically significant, its magnitude in terms of hemostatic significance was marginal.

Bleeding frequency was significantly increased during the danazol phase; 53 episodes occurred during the entire 8 weeks of the danazol phase as compared with 33 episodes occurring during the placebo phase, with a mean of 3.0 ± 2.6 episodes per patient in the Danazol phase vs 1.5 ± 2.4 episodes per patient during the placebo phase (P = .014). It is also noteworthy that bleeding sites had a somewhat different pattern during the two treatment periods, with seven episodes of frank hematuria and one episode of spontaneous oral mucosal bleeding reported during the danazol phase in contrast to only one episode of hematuria and no episodes of spontaneous oral mucosal bleeding in the placebo phase. In particular, patient 2, who only bleeds with trauma, experienced increased and spontaneous bleeding episodes during the danazol phase, including one episode of frank hematuria that was controlled with DDAVP infusion. As a result of increased bleeding during the danazol phase, patients consumed more factor concentrate than in the placebo phase (125 ± 135 U/kg/8 wk vs 71 ± 70 U/kg/8 wk,  P = .024). In individual patients, there was no clear correlation between bleeding episodes and clotting factor levels. Hematologic parameters (Table 2) revealed no significant differences in WBC counts, fibrinogen, or PT between the placebo and danazol periods. A clinically minimal but statistically significant decrease in the hemoglobin level, increase in platelet counts, and shortening APTT were noted during the danazol period. It is not clear whether these effects are related to increased bleeding episodes or the direct effect of danazol. Antithrombin III levels and fibrinolysis parameters are shown in Table 3. It is of note that protein C, plasminogen, and antithrombin III levels were significantly increased during the danazol period (protein C, 111.12% ± 26.22% vs 77.62% ± 15.99%,  P = .0003; plasminogen, 115.33% ± 12.81% vs 100.25% ± 14.95%,  P = .0003; antithrombin III,
EFFECT OF DANAZOL IN HEMOPHILIA

Table 1. Effect of Danazol on Clotting Factor Levels, Bleeding Frequencies, and Amount of Factor Infusion

<table>
<thead>
<tr>
<th>Patient</th>
<th>Factor Deficiency</th>
<th>Factor Level a (Percent Activity)</th>
<th>Bleeding Frequency (Episodes/8 wk)</th>
<th>Factor Infusion (U/kg/B wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>Danazol</td>
<td>Placebo</td>
</tr>
<tr>
<td>1</td>
<td>VIII</td>
<td>6.0</td>
<td>6.4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>VIII</td>
<td>4.9</td>
<td>5.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>VIII</td>
<td>4.5</td>
<td>4.8</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>VIII</td>
<td>2.6</td>
<td>3.5</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>VIII</td>
<td>2.0</td>
<td>2.8</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>VIII</td>
<td>1.8</td>
<td>4.4</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>VIII</td>
<td>1.3</td>
<td>1.5</td>
<td>5 (H x 1)</td>
</tr>
<tr>
<td>8</td>
<td>VIII</td>
<td>0.9</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>VIII</td>
<td>0.8</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>VIII</td>
<td>0.8</td>
<td>0.6</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>VIII</td>
<td>0.5</td>
<td>0.6</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>VIII</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>IX</td>
<td>1.5</td>
<td>2.5</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>IX</td>
<td>1.2</td>
<td>2.1</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>IX</td>
<td>1.1</td>
<td>1.3</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>IX</td>
<td>0.9</td>
<td>1.0</td>
<td>2</td>
</tr>
</tbody>
</table>

Mean ± SD: 2.0 ± 1.98, 2.5 ± 1.88, 1.5 ± 2.4, 3.0 ± 2.6, 71 ± 70, 128 ± 1

P value: .0002, .014, .024

Abbreviations: H, gross hematuria; OM, bleeding from oral mucosa.

*Factor levels are an average of levels drawn on day 3 and weeks 2, 4, and 8 of each of the placebo or danazol phases. Factor levels drawn within three days of concentrate infusion were not included.

†Patient 2 received five separate infusions of DDAVP to control bleeding during the danazol phase.

§Amount of factor infusion not included because this patient received a large amount of clotting factor concentrate for major surgical procedure.

§§Patient 15 received an infusion of factor concentrate for a dental procedure.

This patient’s log for factor infusion is not available.

Liver enzymes were followed because danazol is known to adversely affect liver function, but no significant changes from the baseline values were noted during the danazol phase as compared with the placebo phase (Fig 1). Only one patient showed a moderate and transient increase in liver enzymes during both treatment periods, but more pronounced during the placebo phase. No other significant side effects were reported by our patients.

DISCUSSION

An oral agent that could increase factor VIII or factor IX levels in hemophilic patients would offer advantages over the use of clotting factor concentrates derived from pooled plasma with their attendant risk of transmission of donor infections. Granick et al,29 reported that danazol, an attenuated androgen, is effective in raising plasma levels of factor VIII and IX in patients with hemophilia A and B. However, their observations were based on a very limited number of patients, with variable treatment periods ranging from 2 weeks to 3 months and without parallel of cross-over controls to eliminate placebo effects. Nevertheless, in the context of the current serious concerns regarding blood component

Table 2. Hematologic Profile During Placebo and Danazol Phases in Hemophilics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Ranges</th>
<th>Placebo</th>
<th>Danazol</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.0–16.0</td>
<td>15.2 ± 1.1</td>
<td>14.7 ± 1.0</td>
<td>.02</td>
</tr>
<tr>
<td>Platelets (x 10^5/µL)</td>
<td>140–440</td>
<td>244 ± 82</td>
<td>278 ± 87</td>
<td>.02</td>
</tr>
<tr>
<td>WBC (x 10^3/µL)</td>
<td>4.0–10.0</td>
<td>5.5 ± 2.1</td>
<td>5.9 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>190–400</td>
<td>228 ± 63</td>
<td>256 ± 92</td>
<td>NS</td>
</tr>
<tr>
<td>PT (s)</td>
<td>11.0–13.0</td>
<td>11.4 ± 0.5</td>
<td>11.3 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>25.0–38.0</td>
<td>77.9 ± 17.8</td>
<td>71.8 ± 17.6</td>
<td>.002</td>
</tr>
</tbody>
</table>

Abbreviation: NS, data not statistically significant (P > .05).
therapy, we believe their report presented important preliminary observations as a lead to larger controlled studies.

Our results, based on a randomized, double-blind crossover study in 16 patients, showed that mean plasma factor VIII and IX levels during the 8 weeks of danazol treatment were minimally higher for the patients as a group than during the placebo phase. The extent of this increase, however, although statistically significant, was of marginal hemostatic magnitude. In fact, the increased plasma factor levels observed during the danazol phase may, in part, be due to the fact that patients had significantly increased bleeding frequency resulting in higher clotting factor requirements. In three patients who had not had any factor infusion during the entire study (patients 1, 2, and 15), plasma clotting factor levels during the danazol period were only 0.2% to 0.6% higher than during the placebo phase. In contrast, only one patient (patient 6) had a significant incremental increase in his factor level during the danazol phase (4.4%) compared with the placebo phase (1.8%), but this patient had experienced six bleeding episodes requiring factor concentrate infusion with danazol treatment compared with only two such episodes during the placebo period. Alternatively, a danazol-mediated delay in the half-disappearance of infused clotting factor, suggested by Kasper and Boylen, may account for the increase in clotting factor levels for those patients who had significantly increased bleeding episodes requiring factor infusion.

More important, we believe, was the significantly increased bleeding frequency and factor concentrate usage during the danazol period. Furthermore, the danazol phase was associated with an increased incidence of hematuria and bleeding from oral mucosa, suggestive of increased fibrinolytic activity. One patient (patient 2), who is an infrequent bleeder, had three episodes of hematuria during the danazol phase, but none while receiving placebo. Although Gralnick et al reported decreased factor concentrate infusion during danazol therapy, their observations were limited to three patients who received danazol for variable periods. Reports by Kasper and Boylen, Garewal et al, Ambriz et al, and a randomized study by Nugent et al did not find danazol to have consistent or significant effects on raising plasma levels of factor VIII and IX, but remarked on an increase in frequency and/or severity of bleeding episodes in some of the patients while receiving danazol. Enhancement of fibrinolytic activity and increase in protein C levels has been reported with stanozolol, another anabolic steroid. Similarly, recent reports have suggested that danazol also enhances fibrinolytic activity in hemophilic patients as determined by the euglobulin lysis test. In our patients levels of protein C and plasminogen were significantly increased during the danazol phase. Protein C, a vitamin K-dependent plasma protein, when activated by thrombin exerts an anticoagulant effect through inactivation of activated factors V and VIII. Additionally, activated protein C has been shown to enhance fibrinolysis through, as yet, an unknown mechanism. A protein C-mediated increase in t-PA has been proposed but not consistently demonstrated in animal models. In our patients, no significant difference was noted in t-PA levels between danazol and placebo periods. Although in our patients the ELT showed no change from the baseline during the danazol period, we recognize that ELT is, at best, a crude screening test for plasma fibrinolytic activity. The observed increase in protein C and plasminogen levels during the danazol phase is highly suggestive of danazol-mediated enhanced fibrinolytic activity but needs further substantiation. Furthermore, a direct cause-and-effect relationship

Table 3. Antithrombin III and Fibrinolysis Parameters During Placebo and Danazol Phases in Hemophiliacs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Ranges</th>
<th>Placebo</th>
<th>Danazol</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombin III (mg/dL)</td>
<td>17–30</td>
<td>27.1 ± 2.6</td>
<td>32.5 ± 3.8</td>
<td>.001</td>
</tr>
<tr>
<td>Plasminogen (%)</td>
<td>75–120</td>
<td>100.25 ± 14.95</td>
<td>115.31 ± 12.81</td>
<td>.00003</td>
</tr>
<tr>
<td>Protein C (%)</td>
<td>60–150</td>
<td>77.62 ± 15.99</td>
<td>111.12 ± 26.22</td>
<td>.0003</td>
</tr>
<tr>
<td>t-PA (mg/mL)</td>
<td>12.2 ± 6.1</td>
<td>11.19 ± 6.11</td>
<td>10.11 ± 5.13</td>
<td>NS</td>
</tr>
<tr>
<td>ELT (U)</td>
<td>&lt;3</td>
<td>1.15 ± 0.32</td>
<td>1.23 ± 0.31</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 4. Immunologic Profiles During Placebo and Danazol Phases in Hemophiliacs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (N = 25)</th>
<th>Placebo (N = 16)</th>
<th>Danazol (N = 16)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total B lymphocytes (/µL)</td>
<td>428 ± 87</td>
<td>249 ± 34</td>
<td>279 ± 71</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total T lymphocytes (/µL)</td>
<td>1,308 ± 320</td>
<td>1,630 ± 254</td>
<td>1,541 ± 208</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>T4 cells (/µL)</td>
<td>863 ± 228</td>
<td>564 ± 144</td>
<td>548 ± 148</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>T8 cells (/µL)</td>
<td>512 ± 156</td>
<td>1,050 ± 213</td>
<td>1,017 ± 174</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>T4/T8 ratio</td>
<td>1.79 ± 0.54</td>
<td>0.7 ± 0.34</td>
<td>0.68 ± 0.38</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Natural killer cells (%)</td>
<td>10.2 ± 1.3</td>
<td>7.7 ± 2.6</td>
<td>6.9 ± 1.8</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>PHA response (× 10^5 cpm)</td>
<td>42.6 ± 13.2</td>
<td>38.1 ± 11.3</td>
<td>41.1 ± 5.4</td>
<td>NS</td>
</tr>
<tr>
<td>MLR response (× 10^5 cpm)</td>
<td>2.01 ± 1.11</td>
<td>1.13 ± 4.18</td>
<td>1.30 ± 7.25</td>
<td>NS</td>
</tr>
</tbody>
</table>

*P values from comparisons between the nonhemophilic controls and the hemophiliacs.

Immunologic profile values are from blood samples drawn during week 8 of both the placebo and danazol phases (N = 16). Nonhemophilic controls were obtained from 25 healthy health professionals of comparable age and sex. No statistically significant difference was noted between the placebo and danazol phase in hemophiliacs.
between enhanced fibrinolysis and the increased bleeding frequency during the danazol phase, although conceptually attractive, remains speculative at this time.

Antithrombin III levels were also significantly increased in our patients during the danazol phase, an observation previously reported with danazol. Antithrombin III and protein C both exert an anticoagulant effect, and increased levels of both would be expected to prolong the APTT. Nevertheless, a shortened APTT has recently been reported in hemophilic patients receiving danazol without a consistent parallel increase in factors VIII or IX, and we have also observed a very small, but statistically significant shortening of the APTT during the danazol phase. Garewal et al have suggested that danazol may induce a de novo appearance of an intrinsic coagulation pathway activator bypassing factors VIII and IX. Interestingly, during the danazol phase, there was a decrease in the hemoglobin level and an increase in the platelet count compared with the placebo phase. Danazol has been shown to increase platelet counts in some patients with idiopathic thrombocytopenic purpura (ITP). It is not clear whether in our study increased platelet counts during the danazol phase are due to a similar mechanism as in ITP, possibly immune mediated, or reactive to increased bleeding episodes.

The effect of danazol on the immune system intrigued us. During the last decade, several studies have addressed the effect of androgenic hormones on the immune system, both humoral and cell mediated, in animal models and in patients with autoimmune diseases. It has been suggested that danazol may also exert an immune-mediated effect in immune thrombocytopenic purpura. We and others had noted abnormalities in T lymphocyte numbers and functions in hemophilic patients. Therefore, we undertook a detailed analysis of humoral and cellular immune parameters in our patients to clarify both the baseline immune status of these hemophilic patients and to document the effect, if any, that danazol had on their immune system. The baseline lymphocyte profiles of our patients were in agreement with earlier reports and in general, treatment with danazol produced no significant changes. The total T cell counts remained elevated, and the ratio of functional T cell subsets reversed. The proportion of NK cells enumerated by the Leu-7 antibody remained somewhat depressed. Danazol appears to have no effect on altering this profile. The changed ratio of T cell subsets is reflected by the mitogenic responses with a reduced MLR response. However, the PHA cultures are not significantly affected. Levels of immune globulins IgA, IgG, and IgM and complement components C3 and C4 remained unchanged.

It is of note that liver function parameters did not significantly change during the danazol period, nor were any significant side effects reported by our patients.

Based on our observations, we conclude that danazol, although relatively safe, does not increase plasma levels of factor VIII or IX to a hemostatically significant degree. On the contrary, we and others have observed increased bleeding frequency during danazol therapy. Our data suggest that danazol may enhance the fibrinolytic process through a
significant increase in plasma levels of protein C and plasminogen, which may directly or indirectly account for an increase in bleeding frequency and factor concentrate use. Danazol is, therefore, not a viable alternative to factor concentrate infusion in the treatment of hemophilia. Danazol also does not seem to have an effect on the humoral and cellular immune systems as detected by current methodology.

ACKNOWLEDGMENT

We wish to acknowledge our gratitude to Winthrop Laboratories for providing the danazol and placebo tablets for this study. We are indebted to Joan Eisele and Linda Zodiak for their nursing care; Maria Probst, Thomas Mathew and Bridget Troccoli for their expert coagulation assays; Debra Donetz and Margaret Montalto for their expert immunological analysis; and Rose Prendergast for the preparation of this manuscript.

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