Effect of Prothrombin Complex Concentrates on Factor VIII Inhibitor Levels

By Carol K. Kasper and the Hemophilia Study Group

Factor VIII inhibitor levels were measured on 261 occasions in 75 hemophilia-A inhibitor patients before and after prothrombin complex concentrate infusion at 13 treatment centers. A rise in inhibitor level to at least twice the pretreatment value occurred in 27 treatment episodes (13.5%), in 27 patients (36%). In 16 patients (21%), such an anamnestic immune response occurred with the first treatment. Factors predisposing to anamnestic responses may include patient idiosyncrasy, low pretreatment inhibitor levels, and exposure to concentrate over several days.

IN THE PAST FEW YEARS, several reports have described the use of prothrombin complex concentrates to control hemorrhage in patients with hemophilia-A who have anti-factor-VIII antibodies (inhibitors). Prothrombin complex concentrates contain factors II, IX, and X, and in some brands, also factor VII. Some portion of these factors, or a complex of them,1 or some hitherto unrecognized component may promote hemostasis in patients with inhibitors, either by direct effects on the coagulation system or perhaps by enhancing platelet coagulant activity.2 Success in achieving hemostasis in some patients has been reported with the use of commonly available licensed concentrates such as Konyne.
PROTHROMBIN COMPLEX AND INHIBITORS

(USA), 3, 7 Proplex (USA), 8-12 PPSB (France), 13 and Prothrombinex (Australia), 14 as well as with the use of experimental concentrates, prepared for sole use in patients with inhibitors, and containing an increased amount of activated clotting factors. The latter concentrates include Auto-IX (formerly called Auto-Proplex, USA), 3, 15-17 FEIBA (“Factor-Eight-Inhibitor-Bypassing-Activity,” formerly called Fraction R, Austria), 18-21 and activated PPSB (Australia). 22

Early reports indicated that patients managed with prothrombin complex concentrates did not suffer the anamnestic elevations in anti-factor-VIII antibody levels that would have been expected if they had been treated with products rich in factor-VIII coagulant activity. 3, 6, 8-14 In fact, inhibitor levels often declined during periods when patients were exposed only to prothrombin complex concentrate. As more experience accumulated, however, reports appeared describing rising inhibitor levels in a few patients after administration of prothrombin complex concentrates, 3, 11, 13, 17, 19, 21, 23, 24 including Konyne, Proplex, PPSB Auto-IX, and FEIBA.

Each of the 13 hemophilia treatment centers in the Cooperative Study of Spontaneously Occurring Inhibitors to Factor VIII in Hemophilia-A has had experience with the use of prothrombin complex concentrates in the management of patients with factor-VIII inhibitors. For this report, we pooled our data on such treatment episodes in an attempt to describe the incidence of rises in factor-VIII inhibitor levels after exposure to prothrombin complex concentrate, any characteristics of the patients that might predispose them to such anamnestic responses, and the relationship, if any, between intensity of exposure to the concentrate and the likelihood of an anamnestic response.

MATERIALS AND METHODS

We reviewed the records of all patients with hemophilia-A and an inhibitor who had received prothrombin complex concentrate between 1975 and 1978 at any of the hemophilia centers participating in the Cooperative Study. A few patients have been reported previously. 5, 7, 12, 23 Subjects in the Cooperative Study have signed informed consents for participation in the study and publication of findings, and the study and consent forms have been approved by the appropriate committees of each participating institution.

An evaluable period of prothrombin complex concentrate therapy met the following criteria: (1) no blood product other than prothrombin complex concentrate was given during the treatment period or in the previous 2 mo, and (2) the level of inhibitor was measured before and after treatment. The posttreatment measurement was no earlier than 5 days after the first infusion of concentrate and no later than 120 days after the last infusion. A treatment period might consist of a single infusion on 1 day or multiple infusions over several days or weeks.

Inhibitor levels were measured in Bethesda U/ml in each participating laboratory, using the same method. 5 This method has a standard deviation of approximately 30%. Plasma samples were sent from each participating laboratory to the Reference Laboratory, Jefferson Medical College, Philadelphia, for confirmation of inhibitor measurements. The variation in inhibitor levels between laboratories was only slightly greater than that within laboratories. For the purpose of this report, an increase in inhibitor level was considered notable only if the posttreatment level was 2 or more Bethesda U/ml and was 2 or more times as high as the pretreatment level.

Statistical tests of significance were performed by chi-square analysis of 2 × 2 contingency tables. 26

RESULTS

Incidence of Anamnestic Responses

A total of 261 treatment episodes were evaluable, and 35 were associated with a notable rise in inhibitor levels. Rising inhibitor levels appeared equally likely to be
Table 1. Distribution of 35 Anamnestic Responses During 261 Treatment Episodes in 75 Patients

<table>
<thead>
<tr>
<th>Treatment Episodes per Patient</th>
<th>Number of Patients</th>
<th>Number of Anamnestic Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>4–6</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>7–18</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>48</td>
</tr>
</tbody>
</table>

discovered if the posttreatment inhibitor measurement was made within 30 days of treatment or if it was made later. Anamnestic responses were observed in 26 of 192 treatment periods (16%) in which the posttreatment inhibitor level was measured within 30 days, in 5 of 41 episodes (12%) in which the level was measured between 31 and 60 days after treatment, and in 4 of 28 episodes (14%) in which the level was measured between 61 and 120 days after treatment.

Seventy-five different patients had at least one evaluable period of treatment with prothrombin complex concentrate (Table 1). An anamnestic response was observed on some occasion in 27 patients.

The number of treated patients from each of the 13 participating institutions ranged from 1 to 13, with a median of 7. No anamnestic responses were reported from 3 institutions that treated 1, 3, and 4 patients, respectively. In the remaining 10 institutions, the percentage of patients who experienced anamnestic responses on some occasion ranged from 14% (1 patient in 7) to 62% (8 patients in 13).

As shown in Table 2, anamnestic responses occurred in the first treatment episodes in 16 of 75 patients (21%). Three of eight patients who had an anamnestic response to the first treatment and who were treated a second time also had an anamnestic response to the second treatment. In contrast, only 4 of 35 patients who did not have an anamnestic response to the first treatment with prothrombin complex concentrate, and were treated a second time, had an anamnestic response to the second treatment. The difference in responses to second treatment episodes between patients who did and did not have anamnestic responses to the first treatment episode is significant at the level of $p = 0.05$.

Table 2. Incidence of Anamnestic Responses According to Sequence of Treatment Episodes

<table>
<thead>
<tr>
<th>Sequence of Treatment Episodes</th>
<th>Number of Episodes</th>
<th>Number of Anamnestic Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>First</td>
<td>75</td>
<td>16 (21%)</td>
</tr>
<tr>
<td>Second</td>
<td>46</td>
<td>7 (15%)</td>
</tr>
<tr>
<td>Third</td>
<td>31</td>
<td>4 (13%)</td>
</tr>
<tr>
<td>Fourth</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Fifth</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>Sixth</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>7th–18th</td>
<td>51</td>
<td>4</td>
</tr>
</tbody>
</table>
Whereas the likelihood of an anamnestic response to a given treatment episode decreased as the number of treatment episodes increased (Table 2), multiple treatment episodes afforded more patients the opportunity to have an anamnestic response on some occasion (Table 1).

The relevance of small elevations in the inhibitor level was evaluated by examining the records of 116 patients with inhibitors who had received no transfusion of plasma products for at least 1 yr and whose inhibitor levels had been measured on at least 2 occasions: once 6 or more mo after the last transfusion and again 6 or more mo after the prior measurement. Inhibitor levels during the period of observation fell in 20 patients, remained constant (±50% of the previous value) in 89 patients (of whom 77 had constant inhibitor levels of zero), and rose in 7 patients. Among the latter 7 patients, the level rose to between 150% and 200% of the previous value in 3 patients, rose from 0 to 2 U/ml over a 6-mo interval in 1 patient, and rose from 10.8 U/ml to 24.5 U/ml over a 6-mo interval in 1 patient. In the remaining 2 patients, the rising inhibitor level may have indicated a delayed anamnestic response. One patient with an inhibitor level of 55 U/ml received 3 U of packed unwashed red blood cells, and 13 days later, his inhibitor level was 58 U/ml; 6 mo later, with no intervening therapy, his inhibitor level was 160 U/ml. Another patient with an inhibitor level of 1.4 U/ml received 40,000 factor-VIII U in the form of concentrate and 55 days later his inhibitor level was zero, but 8 mo after the infusion, with no intervening therapy, his inhibitor level was 16 U/ml. Thus, notable rises in inhibitor levels of an apparently spontaneous nature, or possibly due to delayed anamnesis, may occur, but only in rare instances.

Intensity of Anamnestic Responses

In the 35 treatment periods associated with anamnestic responses, the posttreatment inhibitor levels were between 2 and 5 Bethesda U/ml in 11 instances. In the remaining 24 episodes, the posttreatment inhibitor levels, all 5 or more Bethesda U/ml, were between 2 and 2.9 times the pretreatment levels in 7 episodes, between 3 and 4.9 times the pretreatment levels in 3 episodes, between 5 and 9.9 times the pretreatment levels in 5 episodes, and 10 or more times the pretreatment levels in 9 episodes (Fig. 1). A few anamnestic responses were dramatic, for example, rises from 6.6 to 312 U/ml, from 9.5 to 540 U/ml, and from 30 to 1370 U/ml.

When a given patient had more than one anamnestic response, the degree of elevation of the inhibitor level tended to be similar in all instances (Table 3).

Correlation With Pretreatment Inhibitor Levels

Pretreatment inhibitor levels tended to be lower in treatment periods, resulting in increased inhibitor levels, than in treatment periods without anamnestic responses (Table 4). Anamnestic responses occurred in 20% of 145 treatment episodes in which pretreatment inhibitor levels were less than 10 U/ml and in 5% of 116 episodes in which pretreatment levels were 10 or more U/ml (p < 0.005). Pretreatment inhibitor levels in those 19 patients who had at least one treatment period with and one without anamnestic response are illustrated in Fig. 2. In most cases, anamnestic responses occurred only when pretreatment inhibitor levels were similar to, or lower than, the lowest level recorded prior to a treatment episode not associated with an anamnestic response.
Fig. 1. Pre- and posttreatment inhibitor levels are illustrated for 35 treatment episodes associated with anamnestic responses. The diagonal line indicates the level at which posttreatment inhibitor measurements are twice the pretreatment values.

Patient History

We could find no correlation between the patient's age at detection of an inhibitor, or his age at treatment with prothrombin complex concentrate, or his prior degree of anamnestic response to factor-VIII infusions and the likelihood of anamnestic response to infusions of prothrombin complex concentrate. Among 48 patients who did not have an anamnestic response to prothrombin complex concentrate...

Table 3. Comparison of Degree of Anamnestic Response in Seven Patients Who Had More Than One Such Response to Prothrombin Complex Concentrate

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>4</td>
<td>1.5</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
<td>2</td>
<td>1.3</td>
<td>4.3</td>
<td>2</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
<td>3.6</td>
<td>3.2</td>
<td>7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.9</td>
<td>4</td>
<td>4.8</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>13.5</td>
<td>3.4</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>16</td>
<td>8.3</td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>150</td>
<td>9.5</td>
<td>540</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Relationship of Pretreatment Inhibitor Levels and Frequency of Anamnestic Responses

<table>
<thead>
<tr>
<th>Pretreatment Inhibitor Levels</th>
<th>Without Anamnestic Response</th>
<th>With Anamnestic Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2 U/ml</td>
<td>44</td>
<td>12 (21%)</td>
</tr>
<tr>
<td>2-4.9 U/ml</td>
<td>28</td>
<td>8 (22%)</td>
</tr>
<tr>
<td>5-9.9 U/ml</td>
<td>44</td>
<td>9 (17%)</td>
</tr>
<tr>
<td>10-29 U/ml</td>
<td>63</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>≥30 U/ml</td>
<td>47</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

concentrate, 45 had histories of marked anamnestic responses to factor-VIII infusions, 1 had a history of little or no anamnestic response to factor VIII, and in 3 patients, the history was unknown. Among 27 patients who did have an anamnestic response to prothrombin complex concentrate on some occasion, 21 had histories of marked anamnestic responses to factor-VIII infusions, 5 did not have such

Fig. 2. Pretreatment inhibitor levels for every evaluable treatment episode are illustrated for 19 patients 'A' through 'S,' each of whom had at least one treatment episode with and one without an anamnestic response. Closed circles indicate inhibitor levels before treatment periods associated with anamnestic responses, and open circles indicate inhibitor levels before treatment periods not associated with anamnestic responses. Vertical lines connect values pertaining to the same patient.
histories, and in 1 patient the history was not available. The five patients who had anamnestic responses to prothrombin complex concentrate infusion but had no histories of marked responses to factor-VIII infusion deserve further comment. One patient had mild responses to both factor-VIII concentrate and prothrombin complex concentrate, with maximum inhibitor levels of 2 Bethesda U/ml. In three patients, the inhibitor level was low at the time of diagnosis, and no further factor-VIII therapy was given; these patients did not have an opportunity to demonstrate whether they could develop marked anamnestic responses to factor-VIII therapy. The fifth patient had a low inhibitor level on diagnosis and was treated intensively for 1-mo thereafter with factor-VIII concentrate without an anamnestic response; he received no blood products in the 5-yr interval between that intensive therapy and his first exposure to prothrombin complex concentrate, which resulted in a marked anamnestic response.

**Concentrate Dosage and Identity**

We looked for a relationship between the intensity of exposure to prothrombin complex concentrate and the occurrence of an immune response. The duration of exposure to the concentrate tended to be longer in treatment episodes associated with an anamnestic response. The number of exposure days was 1–3 in 65% of episodes not associated with an anamnestic response and 49% of episodes associated with an anamnestic response ($p < 0.1$). The number of exposure days exceeded 6 in 14% of episodes not associated with an anamnestic response and 34% of episodes associated with an anamnestic response ($p < 0.01$).

We could find no difference in the typical daily dose of concentrate during treatment periods associated with anamnestic responses and those without such responses either in individual patients who had both types of experiences or in the entire subject group when patients of similar weights were compared.

The brand of concentrate was not related to the likelihood of an anamnestic response. Such a response was observed in 21 of 125 treatment episodes (14%) in which Konyne alone was used, in 13 of 76 episodes (17%) in which Proplex alone was used, in 1 of 11 episodes (9%) in which Auto-IX alone was used, and in 0 of 15 episodes in which more than one brand of concentrate was used.

A great many different lots of each brand of concentrate were used, and rarely was a given lot used in several different subjects. We were not able to estimate whether any one lot was more likely than another to be associated with an anamnestic immune response.

**DISCUSSION**

A rise in factor-VIII inhibitor level is an occasional consequence of prothrombin complex concentrate infusion in patients with hemophilia-A and factor-VIII inhibitors. We sought the answers to the following questions: (1) How frequent are anamnestic responses to prothrombin complex concentrates? (2) Do patients show antibody responses characteristic of the individual patient? (3) Is the intensity of exposure to concentrate related to the likelihood of anamnesis? (4) Do specific brands or lots of concentrate differ in their ability to stimulate inhibitor production?
Incidence of Anamnestic Response

In our series, an anamnestic response was experienced at least once in 27 of 75 patients (36%) and in 35 of 261 evaluable treatment episodes (13%). The latter figure may have been biased by the tendency of those patients not having an anamnestic response early in their course to have many episodes of treatment with prothrombin complex concentrate, and the tendency of patients having an anamnestic response, especially if it was of a marked degree or occurred early in their course, to have no further treatment with the concentrate. A spontaneous rise of inhibitor concentration to 2 or more U/ml, and twice or more the previous value, was observed in only 1 of 116 nontransfused patients followed for 6 mo or more.

Observers who have previously reported smaller series of patients have had varying experience with inhibitor response. Anamnestic responses were not encountered at all by several investigators, including Abildgaard et al.,3 who used Konyne to treat 64 bleeding episodes in 5 patients; Kelly and Penner,4 who used Proplex to treat 90 bleeding episodes in 13 patients; Yolken and Hilgartner,12 who used Proplex to treat 43 bleeding episodes in 9 patients; and Price et al.,14 who treated 3 patients with Prothrombinex. Anamnestic responses were reported in a few patients by other investigators, including Kasper and Feinstein,23 who encountered rising inhibitor levels in 4 of 7 patients treated with Konyne; Lusher et al.,7 who observed such responses on one or more occasions in 3 of their 5 patients treated with Konyne; Allain and Krieger,13 who reported anamnestic responses to PPSB in 2 patients; Mannucci et al.,19 who noted marked increases in inhibitor levels in 4 of 5 patients managed with Fraction R (an early form of FEIBA); Preston et al.,21 who encountered an anamnestic response in 1 of 3 patients treated with FEIBA; and Deutsch et al.,24 who observed rising inhibitor levels on 6 of 31 occasions in 5 of 10 patients given FEIBA. These differences in the experience of various clinicians may be due to chance alone, for the number of patients with inhibitors at any one center is small.

Susceptibility of Individual Patients

We noted that an anamnestic response was more likely to occur during the first treatment with prothrombin complex concentrate than during the second if the first episode had been free of immune response. Eight of the 13 most marked instances of immune response, with posttreatment inhibitor levels more than 5 times as high as pretreatment levels, occurred during first exposures to prothrombin complex concentrate, and 3 instances occurred during second exposures of patients who had had marked responses to first exposures. Less marked anamnestic responses tended to be scattered among other patients, some of whom were treated often and had anamnestic responses on a small minority of occasions. These observations suggest that a few patients may have a special sensitivity to prothrombin complex concentrate, whereas the majority of patients have less susceptibility and may have anamnestic responses, usually of a minor degree, only when other relevant predisposing circumstances are present.

Effect of the Pretreatment Inhibitor Level

In our series, we noted that anamnesis was more likely to occur when pretreatment inhibitor levels were relatively low. Although inhibitor levels have been
measured by various methods in published reports of anamnestic responses from other investigators, the pretreatment levels they described tended to be low, in agreement with our observations. Three observers used the Oxford inhibitor unit developed by Drs. Rizza and Biggs: Price et al.\textsuperscript{14} reported anamnestic responses in 3 patients whose baseline inhibitor levels were 3, 3.2, and 4 Rizza-Biggs U/ml; Preston et al.\textsuperscript{31} reported an elevation of the inhibitor level in their patient with a baseline inhibitor level of 14 Rizza-Biggs U/ml but no rise in inhibitor levels in patients with baseline values of 250 and 1080 U/ml; Mannucci et al.\textsuperscript{19} reported anamnestic responses in patients with pretreatment inhibitor levels 0.5, 3.4, and 9 Rizza-Biggs U/ml but no such response in one patient with a baseline level of 0.7 U/ml. Kelly and Penner\textsuperscript{8} used an assay similar to the Bethesda method; no anamnestic responses were noted in any of their patients whose baseline inhibitor levels ranged from 5 to 8 U/ml on 5 occasions and from 13 to 680 U/ml on 9 occasions. Allain and Krieger\textsuperscript{13} reported anamnestic responses in 2 patients whose baseline inhibitor levels of 1.8 and 0.2 U/ml were measured by an assay similar to the Bethesda method, with the exception that a unit of antibody was defined as inactivation of 75\% (instead of 50\%) of the factor VIII in the incubation mixture at 2 hr.

Perhaps patients whose inhibitor levels were high were able to effectively destroy any infused factor VIII, if factor VIII is indeed the provocative agent, before it reached immunocytes; or perhaps these patients were already producing antibody at their maximum current capacity.

**Effect of Intensity of Exposure and Identity of Concentrate**

Our data showed that exposure to prothrombin complex concentrate on several days was associated with a higher incidence of anamnestic responses than exposure on only 1, 2, or 3 days. In contrast, the dose of concentrate per exposure day did not correlate with the likelihood of an anamnestic response.

All three brands of concentrate used in the patients in this series were associated with similar incidences of anamnestic responses. Such responses have been reported with the use of several other brands of concentrate in other countries.

**CONCLUSION**

Anamnestic responses following the use of prothrombin complex concentrate in patients with factor-VIII inhibitors have now been observed in many hemophilia treatment centers and must be regarded as a definite risk of this mode of therapy. Certain patients may have a greater inherent susceptibility to antibody stimulation by prothrombin complex concentrate than others. The likelihood of an anamnestic response appears to be greater if the following conditions exist: (1) the patient has had a previous moderate to marked anamnestic response to prothrombin complex concentrate; (2) the patient’s pretreatment inhibitor level is relatively low for him; (3) the concentrate is administered over several days.

The reason for anamnestic responses of anti-factor-VIII inhibitor on exposure to prothrombin complex concentrate is uncertain. The concentrate may be contaminated with factor VIII. Trace amounts of a substance with partial identity to factor-VIII-related antigen was demonstrated by immunodiffusion in Konyne,\textsuperscript{23} and small amounts of factor-VIII-related antigen also were detected by immunonora-
diometric assay in single lots of Konyne, Proplex, PPSB, FEIBA, and very faintly, in Auto-IX. In the latter study, the amount of antigen varied 100-fold from the least to the most contaminated material. Recently, substantial amounts of factor-VIII procoagulant antigen have been detected by immunologic tests with antibodies sensitive to the coagulant entity in two brands of concentrate available in this country.

In the Inhibitor Study Group, we reached a consensus that each of us would prefer to treat an inhibitor patient with a life-threatening hemorrhage with factor-VIII concentrates if the inhibitor level were low enough to make such management feasible. There is a consensus that factor-VIII concentrates are more reliable and predictable in obtaining hemostasis in the absence of inhibitor than prothrombin complex concentrates are in the presence of inhibitor. We prefer, therefore, to keep inhibitor levels as low as possible in our patients with inhibitors. Most of us continue to use prothrombin complex concentrate to treat hemorrhages in patients with factor-VIII inhibitors, but we are concerned that these concentrates can cause a rise in inhibitor levels in some patients and under some circumstances. We hope that the mechanism by which prothrombin complex concentrates stimulate anamnestic reactions in certain patients will be elucidated. If trace amounts of factor VIII are responsible for the observed effect, perhaps a way may be found to decrease the contamination of prothrombin complex concentrates with factor VIII. In the interim, perhaps those lots with the least contamination may be identified for use in patients with inhibitors.

REFERENCES


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CK Kasper