Immune Tolerance Therapy for Haemophilia A Patients with Acquired Factor VIII Alloantibodies: Comprehensive Analysis of Experience at a Single Institution


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Summary

Eleven children with severe haemophilia A associated with the IVS 22 inversion and acquired high titre neutralising antibodies to factor VIII underwent immune tolerance induction. HLA class I and high resolution class II type is detailed for each patient. A three phase approach to immune tolerance induction was used. During phase 1, which lasted a median of six weeks, patients received factor VIII 100 IU/kg twice daily. Phase 2 comprised a factor VIII dose reduction to 100 IU/kg once daily, and continued for a median duration of 14 weeks. Subsequently 10 of the 11 patients satisfied the criteria of absent factor VIII neutralising activity by the Bethesda method, and a factor VIII elimination half life of greater than 5 h, allowing progression to phase 3, a further factor VIII dose reduction to 50 IU/kg three times weekly. A model for dose reduction as factor VIII tolerance evolves, based on pharmacokinetic analysis, is described.

Introduction

The development of factor VIII neutralising alloantibodies during replacement therapy in patients with severe haemophilia A is a treatment complication with substantial clinical and financial implications. Detected after a median of nine to 36 exposure days, the incidence of neutralising antibody development ranges from 15.6% to 28% in prospective studies of both plasma-derived and recombinant products in previously untreated patients (1-3). Inhibitory antibodies are predominantly of the IgG4 subclass (4), and often demonstrate specificity restricted to the C2 domain of the factor VIII light chain and/or the A2 domain of the heavy chain (5, 6). Low titre antibodies (less than 5 Bethesda units/ml) may spontaneously regress. High titre (greater than 10 Bethesda units/ml) and/or high responding factor VIII inhibitory antibodies, where the titre exhibits an anamnestic response to further factor VIII exposure, preclude factor VIII therapy at standard replacement doses (7, 8). Therapy directed at controlling haemorrhage or eradicating the inhibitor may incur substantial and unpredictable financial costs. We now report a thorough analysis of the management of 11 children with severe haemophilia A and inhibitory antibodies to factor VIII at our comprehensive care haemophilia centre. Careful attention to the timing of initiation of an immune tolerance programme together with appropriate pharmacokinetic analysis of immune tolerance induction allows a prophylactic factor VIII replacement programme to be re-established in a highly cost-effective manner.

Patients and Methods

Patients and immune tolerance programme. All children with severe haemophilia A (factor VIII:C less than 1 IU/dl) registered at our centre are offered continuous prophylaxis with factor VIII replacement. This report describes the management of all children at our centre presenting with a factor VIII inhibitor over a 10 year period from 1987 to 1997. In all cases parents were counselled regarding therapeutic options, and consented to their children entering into a high dose immune tolerance induction (ITI) programme. During the preliminary phase of the immune tolerance protocol, prior to high dose factor VIII administration, bleeding episodes were managed with inhibitor bypassing agents, either FEIBA® (Immuno) or, subsequent to its licensing in the UK, recombinant factor VIIa (NovoSeven®, Novo Nordisk). Wherever possible, commencement of high dose factor VIII treatment was deferred until the neutralising antibody titre had declined to less than 10 or ideally less than 5 Bethesda units/ml. When this criterion was achieved, phase 1 (factor VIII 200 IU/kg/day) of the immune tolerance programme commenced. Phase 2 was defined as a dose reduction to 100 IU/kg as a daily intravenous bolus dose, and commenced after a phase 1 duration of between 4 and 12 weeks, when the neutralising antibody was undetectable by the Bethesda technique (our laboratory reports a Bethesda value of less than 1 BU/ml as negative). Phase 3 was defined as prophylactic factor VIII replacement at a dose of up to 50 IU/kg three times per week. Phase 3 commenced when pharmacokinetic analysis documented a factor VIII elimination half life of greater than five-to-six hours.

Factor VIII assay and neutralising antibody detection. Factor VIII procoagulant activity was measured on citrated plasma by a one stage clot based assay using a KC10 coagulometer. Neutralising antibodies to factor VIII were assessed on citrated plasma on a KC10 coagulometer by the Bethesda technique (9).

Factor VIII-binding assay. The radioimmuno-precipitation assay (RIPA) for detecting antibodies to factor VIII epitopes was a modification of the sensitive assay developed by Prescott et al. (10). Highly purified recombinant factor VIII (rFVIII) was generously provided by Dr. R. W. Kuhn (Bayer Corporation, Berkeley, CA, USA). Its specific activity was approximately 5,000 U/mg when rFVIII activity was measured in a one-stage clotting assay. 30 µg of rFVIII in 0.2 M Na Acetate, 5 mM Ca(NO3)2, was radiolabelled by incubation with 37 MBq of Na125I (Amersham International), 10 µl of Lactoperoxidase beads (Worthington Biochemicals) for 10 min at room temperature. Free Na125I was removed on a PD10 column (Pharmacia Biotech). 125I-rFVIII had a specific activity of 33.33 kBq/µg. 125I-rFVIII was diluted to 1.6 µg/ml in 20 mM Tris, 150 mM NaCl, 1% BSA, pH 7.4 (TBS-BSA), 10 µl (approx. 30,000 cpm) was added to 50 µl of diluted plasma (1:2 in TBS-BSA) in duplicate and incubated at 4° C for 18-20 h. The 125I-rFVIII-anti-rFVIII immune complexes were then precipitated by the addition of 150 µl of 1 in 3 dilution of protein G Sepharose (Pharmacia Biotech) in TBS-BSA with incubation on a rotary shaker for 3 h at 4° C. The precipitate was then washed 3 times with 1 ml of TBS, 0.05% Tween 20 (Bio-Rad Laboratories) with brief centrifugation to pellet the Protein G Sepharose. The amount of 125I-rFVIII precipitated (‘bound’ activity) was then quantified by counting on a gamma counter. Non-specific activity was assessed from a buffer control. The results are represented as % bind-

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ing: [(Bound minus Non-specific activity)/Total] × 100. A normal range for this technique of less than 0.96% was established by processing plasma samples from eighty four healthy blood donors. The mean % binding in this group was 0.104%, standard deviation 0.43%, mean coefficient of variability 19%.

A europium-based non-radioactive method for the detection of factor VIII binding antibody was developed. Lyophilised recombinant factor VIII (Recombinant™, Baxter Hyland®) was dissolved in buffer (NaHCO₃ 0.1 M mixed with Na₂CO₃ 0.1 M to pH 9.6) to 25 IU/ml and incubated in microtitre plates at 4°C overnight. After four washes with buffer in an automated plate washer, wells were blocked with 1% BSA (Sigma) for 30 min at room temperature and washed again. Dilutions of patient and control plasmas were then incubated in wells at 4°C overnight. After four further washes, 100 µl of europium labelled anti-human IgG (EG & G Wallac), diluted 1 in 500, was added to each well and incubated at 4°C overnight. Plates were then washed four times in wash concentrate and 200 µl of enhancing solution (EG & G Wallac) added to each well. After storage in the dark for 10 min, plates were read using a time-resolved fluorescence reader (Arcus, EG & G Wallac) and reported in Time Resolved Fluorescence (TRF) units. A normal range for this technique of less than 39.4 × 10³ TRF units was established by processing plasma samples from eighty seven healthy blood donors. The mean factor VIII-binding activity in this group was 20 × 10³ TRF units, standard deviation 9.7 × 10³ TRF units, mean coefficient of variability 8%.

**Pharmacokinetic analysis.** Studies of factor VIII pharmacokinetics were performed during and at intervals after high dose factor VIII therapy for induction of immune tolerance. After a four day washout period, blood samples were taken before and at intervals after a 50 IU/kg factor VIII bolus dose, until measured factor VIII procoagulant activity had returned to native baseline levels. The factor VIII elimination half life (t½) was assessed using a pharmacokinetic analytical programme (PKAnalyst, MicroMath® Scientific Software, Salt Lake City, UT), with data fitting based on a two compartment model.

**Statistical analysis.** Correlation coefficients between t½ values and factor VIII binding by the europium based assay and by the RIPA method were calculated using the Pearson’s product-moment correlation.

**IVS 22 inversion method and HLA typing.** DNA was extracted from 1-3 ml of whole blood by standard techniques (Puregene™, Flongen, UK). DNA (8 µg) was digested with Bcl I (Promega, Madison, USA) for 6 h at 50°C, and electrophoresed on a 0.5% agarose gel (Promega, Madison, USA) at 80 v overnight before being Southern blotted onto Hybond N (Amersham, UK) and UV cross-linked. Filters were prehybridized in 2 × SSC, 2 Denhardt's, 0.5% SDS, and 100 µg/ml Geneblock (Advanced Biotechnologies, Epsom, UK) at 68°C for 2 h, and then hybridized to a 3P labelled (Multiprimer DNA labelling kit, Amersham, UK) EcoRI/Sac I fragment of p482.6 in 2 × SSC, 0.5% SDS, 100 µg/ml Geneblock for 4 h at 68°C. Filters were washed in 0.2 × SSC, 0.1% SDS at 65°C for 30 min, then 0.2 × SSC for 30 min prior to autoradiography (11). EDTA blood samples were collected for tissue typing at the National Blood Service reference laboratory. Low resolution class I typing was performed by serological methods. High resolution class II typing was performed by PCR-SSP.

**Results**

As a result of either routine three monthly screening for neutralising factor VIII antibodies or of investigation into reduced haemostatic control, 11 children, most with high titre inhibitors, were identified between January 1987 and December 1996. The patients had a median age of 2 years (range 0.8-4.5 years) at the time of inhibitor detection, after a median of 20 exposure days (range 4-37). Patient demographic details are shown in Table 1. Most children were of British Caucasian origin, except patients 2 and 10, who were of African extraction and Asian extraction respectively. Only two patients had a documented maximum inhibitor titre of less than 10 BU/ml (patients 1 and 9). In both these cases, the potential for anamnestic response in inhibitor titre was avoided by immediate conversion to recombinant factor VIII as a therapeutic modality to control bleeding. Molecular analysis confirmed that the IVS 22 inversion was the causative mutation in all cases. HLA genotype of each patient is shown in Table 2. Patients 6 and 7 were brothers, and HLA identical. All 11 consecutive patients proceeded to an IT programme, the details of which are shown in Table 3. Unless the clinical condition necessitated earlier intervention, phase 1 of the programme was delayed until the inhibitor titre had declined to as low a level as possible. In most cases the inhibitor titre at the onset of phase 1 was less than 5 BU/ml (median 2.9, range 0-6.20). Five patients (ID 2, 5, 6, 7 and 8) were diagnosed with a factor VIII inhibitory antibody prior to 1994, when our centre started to perform immune tolerance induction procedures. These 5 patients had delays of 6.5, 4.0, 5.5, 2.5 and 3.5 years respectively between diagnosis and start of immune tolerance induction. Other patients had a median interval of 3 weeks (range 2-52) between inhibitor diagnosis and start of immune tolerance induction. There were six patients receiving monoclonal purified, high purity, plasma derived factor VIII concentrate at the time of inhibitor diagnosis. Immune tolerance was established with the same plasma derived product in one of these (patient 5) and with a recombinant product in the other five (patients 2, 3, 6, 7 and 8). The other five patients (1, 4, 9, 10 and 11) who had always received recombinant factor VIII were tolerised with the same product. Phase 1 commenced with continuous factor VIII infusion to provide perioperative haemostatic control for inser-
tion of a central venous access device. With this regime, measured factor VIII binding was continued on daily factor VIII prophylaxis (2000 IU) for thrombotic complications, related to central lines in any of the 11 patients (p = 0.016). There were no adverse events, in particular no infective or allergic reactions, as assessed by both the RIPA test and the Time Resolved Fluorescence (TRF) method. The two methods of detecting factor VIII binding activity were moved to phase 3 without clinical deterioration. Patients 2 and 9 were continued on daily treatment despite suitable t1/2 values. Patient 10, who commenced phase 1 with an inhibitor titre of 20 BU/ml, required a factor VIII infusion of 50 IU/kg/h to achieve this. Phase 1 was a median of 6 weeks duration (range 4-12). The timing of dose reduction to phase 2 (from twice daily to once daily factor VIII 100 IU/kg) was based on the inhibitory antibody titre being undetectable by the Bethesda technique and measured factor VIII recovery of greater than 60-80%. Pharmacokinetic assessment was an important consideration in the timing of dose reduction from phase 2 to phase 3 (factor VIII 100 IU/kg/day to 50 IU/kg three times per week). Pharmacokinetic analysis performed at 8-12 weeks into phase 2 showed a factor VIII t1/2 of at least 5 h in all cases except patient 7, whose t1/2 was 0.2 h. Patient 7 was continued on daily factor VIII prophylaxis (2000 IU) for 30 months before his t1/2 increased from 1.8 to 4.5 h, at which time he was moved to phase 3 without clinical deterioration. Patients 2 and 9 were continued on daily treatment despite suitable t1/2 for a total of 22 and 20 weeks respectively to allow inflammation in target joints to fully settle in the absence of bleeding. Results of factor VIII binding studies, as assessed by both the RIPA test and the Time Resolved Fluorescence (TRF) method, on samples taken at variable time points in phase 3 of the ITI programme are shown in Table 4, together with corresponding RIPA results. These samples all showed no detectable inhibitor by the Bethesda technique. The t1/2 values showed a correlation coefficient of -0.498 (p = 0.119) with % alloantibody binding by the RIPA technique, and a correlation coefficient of -0.528 (p = 0.117) with factor VIII binding by the TRF method. The two methods of detecting factor VIII binding activity, RIPA and TRF, showed a correlation coefficient of 0.734 (p = 0.016). There were no adverse events, in particular no infective or thrombotic complications, related to central lines in any of the 11 patients. All boys continue prophylactic factor VIII replacement therapy.

### Discussion

The pathogenesis of development of neutralising factor VIII antibodies in haemophilia A patients is unclear. Attempts at defining the immune responsiveness of haemophiliacs through documentation of HLA type have not yet identified strong predictors for inhibitor development (12), possibly because of the high degree of heterogeneity of both class II HLA type and the individual genetic mutations causing haemophilia. Oldenburg et al. (13) studied a group of severe haemophilia A patients with a single causative mutation, inversion of IVS 22, and determined HLA genotype. They found the haplotype HLA-A3, B7, C7, DQA0102, DQB0062. DR15 was weakly correlated to inhibitory antibody titre being undetectable by the Bethesda technique and measured factor VIII recovery of greater than 60-80%. Pharmacokinetic assessment was an important consideration in the timing of dose reduction from phase 2 to phase 3 (factor VIII 100 IU/kg/day to 50 IU/kg three times per week). Pharmacokinetic analysis performed at 8-12 weeks into phase 2 showed a factor VIII t1/2 of at least 5 h in all cases except patient 7, whose t1/2 was 0.2 h. Patient 7 was continued on daily factor VIII prophylaxis (2000 IU) for 30 months before his t1/2 increased from 1.8 to 4.5 h, at which time he was moved to phase 3 without clinical deterioration. Patients 2 and 9 were continued on daily treatment despite suitable t1/2 for a total of 22 and 20 weeks respectively to allow inflammation in target joints to fully settle in the absence of bleeding. Results of factor VIII binding studies, as assessed by both the RIPA test and the Time Resolved Fluorescence (TRF) method, on samples taken at variable time points in phase 3 of the ITI programme are shown in Table 4, together with corresponding RIPA results. These samples all showed no detectable inhibitor by the Bethesda technique. The t1/2 values showed a correlation coefficient of -0.498 (p = 0.119) with % alloantibody binding by the RIPA technique, and a correlation coefficient of -0.528 (p = 0.117) with factor VIII binding by the TRF method. The two methods of detecting factor VIII binding activity, RIPA and TRF, showed a correlation coefficient of 0.734 (p = 0.016). There were no adverse events, in particular no infective or thrombotic complications, related to central lines in any of the 11 patients. All boys continue prophylactic factor VIII replacement therapy.

### Table 4

<table>
<thead>
<tr>
<th>Patient</th>
<th>ITI on phase 3 (years)</th>
<th>Current Factor VIII</th>
<th>RIPA (% binding)</th>
<th>Factor VIII binding (TRF units x 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3</td>
<td>5.0</td>
<td>58</td>
<td>0.46</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>10.2</td>
<td>80</td>
<td>0.46</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>12.1</td>
<td>98</td>
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<tr>
<td>4</td>
<td>3.3</td>
<td>8.0</td>
<td>60</td>
<td>0.18</td>
</tr>
<tr>
<td>5</td>
<td>3.5</td>
<td>8.6</td>
<td>92</td>
<td>3.59</td>
</tr>
<tr>
<td>6</td>
<td>1.8</td>
<td>10.4</td>
<td>80</td>
<td>1.70</td>
</tr>
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<td>7</td>
<td>4.3</td>
<td>4.5</td>
<td>41</td>
<td>3.32</td>
</tr>
<tr>
<td>8</td>
<td>2.4</td>
<td>8.6</td>
<td>100</td>
<td>0.00</td>
</tr>
<tr>
<td>9</td>
<td>0.8</td>
<td>12.7</td>
<td>67</td>
<td>1.9</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>7.6</td>
<td>54</td>
<td>7.72</td>
</tr>
<tr>
<td>11</td>
<td>1.9</td>
<td>7.6</td>
<td>105</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Note: Time Resolved Fluorescence (TRF) techniques. na: not available; NR: normal range.
Presence of both factor VIII-binding and specific anti-idiotypic antibodies have been documented in desensitised inhibitor patients (19) and people without haemophilia (20), and do not necessarily reflect factor VIII neutralising activity. We have presented factor VIII binding results as assessed by RIPA and Time Resolved Fluorescence techniques, together with corresponding pharmacokinetic data, and showed that these techniques can provide complimentary information on the biological fate of infused factor VIII. The two methods measuring factor VIII binding activity were positively correlated with each other and negatively correlated with $t_{1/2}$, although study of a larger inhibitor patient group would be required to demonstrate statistical significance.

In conclusion our findings provide a framework for development of dose adjustment guidelines throughout an ITI programme, based on pharmacokinetic assessment. Factor VIII binding assays using the RIPA and/or Time Resolved Fluorescence techniques may provide useful information, particularly if performed serially, and interpreted in conjunction with pharmacokinetic data. A comprehensive approach to induction of immune tolerance, including systematic assessment of impact on quality of life is essential to justify the cost effectiveness of ITI programmes.

References


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