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Immune Tolerance Therapy for Haemophilia

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Abstract

The development of anti-factor VIII and anti-factor IX allo-antibodies in haemophilia A and B, respectively, remains a serious complication of treatment for these two X-linked haemostatic disorders, with major clinical and economic consequences. Treatment of this potentially fatal complication remains one of the greatest challenges facing haematologists at the beginning of the 21st century. Immune tolerance induction (ITI) therapy has been generally accepted as the best available treatment, extinguishing the inhibitor and permitting a resumption of standard dosing schedules. Although there have been several established protocols for ITI therapy developed over the last quarter century, the optimal scheme in terms of safety, clinical efficacy and pharmacoeconomic considerations has yet to be determined.

Allo-inhibitors to factor VIII (FVIII) and factor IX (FIX) in patients with haemophilia A and B, respectively, are a major complication of replacement therapy. Inhibitors are immunoglobulin (Ig)G antibodies formed against specific epitopes of the FVIII or FIX molecule, and may neutralise the procoagulant properties or reduce the recovery and half-life of infused FVIII or FIX. Patients with high titre inhibitors are refractory to replacement therapy and at risk of severe uncontrollable bleeding with associated morbidity and mortality. FVIII autoantibodies in patients with 'acquired' haemophilia A[1] occur de novo or secondary to autoimmune disease, pregnancy and lymphoid malignancies, and are extremely rare. FVIII allo-inhibitors in haemophilia A form the focus of this review, since FIX allo-inhibitors in patients with haemophilia B are relatively uncommon (incidence 1.5 to 3% overall).[2]

1. Epidemiology

Kreuz et al.[3] recently reviewed the epidemiology of inhibitors in patients with haemophilia A and described an overall prevalence of 7 to 18% according to population and study design, with an overall incidence of 18.4 to 28%. Not surprisingly, the incidence was highest in patients with severe haemophilia A [FVIII coagulant activity (FVIIIc) <2%]. However, inhibitors were not uncommon in patients with moderate (FVIIIc 2 to 5%) and mild (FVIIIc >5%) disease. Inhibitors typically develop in patients with mild/moderate haemophilia A following intense replacement therapy and are usually of low titre, but they may be problematic as they neutralise endogenous FVIII. The prevalence of inhibitors in these patients is thought to be between 3 and 13%,[4-6] with an annual incidence of 0.84 per 1000 patients per year.[7]

Lusher et al.[4] reported a total incidence of 19.7% in previously untreated patients (PUPs), with incidences of 28.5, 13.3 and 0% in patients with severe, moderate and mild haemophilia A, respectively, receiving recombinant FVIII (Kogen-

ate®).[1] More recently, Rothschild et al.[8] described an inhibitor incidence of 28% in PUPs with severe haemophilia A receiving recombinant FVIII (Recombinate®),[1] mostly appearing within 25 cumulative exposure days. However, inhibitors have been detected as early as 10 to 13 days following birth and exposure to exogenous FVIII.[9] The use of moroctocog alfa (B domain deleted recombinant FVIII) appears to be associated with an incidence of approximately 30%.[10] In severe haemophilia, the recent introduction of routine prophylaxis in children and increased surveillance has resulted in a demographic shift of inhibitors to a younger population of patients, with implications for clinical management and the interpretation of data with studies using historical comparisons.[11]

There have been several studies attempting to identify risk factors for inhibitor development. Large deletions, nonsense mutations or inversions (e.g. IVS 22 inversion) are the most frequent FVIII gene mutations in patients with inhibitors.[12] IVS 22 inversion in severe haemophilia A has been identified as a significant risk factor in some studies[7,13,14] but not others.[15,16] In moderate and mild haemophilia, mutations affecting the A2 and C2 domains appear to be common in patients with inhibitors, although mutations affecting the A1, A3 and C1 domains have also been reported.[7]

Evidence for a genetic predisposition to inhibitor formation is apparent. Family studies demonstrate that the incidence of inhibitor development in siblings with haemophilia and members of the extended family is approximately 50 and 11%, respectively.[17] Both concordance and discordance for inhibitor development between monozygotic twins have been described.[18,19] Ethnic variation is apparent,[17] and although no strong HLA-linkage has been identified, several studies suggest associations. In patients with severe haemophilia with IVS 22 inversion, the haplotypes HLA-A3, B7, C7, DQB0602, DR15 demonstrate a weak correlation with inhibitor development.[20] This finding was confirmed, and DRB1*1501, DQA1*0102 and DQB1*0602 was also identified as a conserved haplotype in 36% of patients with inhibitors.[21]

The association of inhibitors with exposure to specific products is attributed to enhanced antigenicity related to changes in the tertiary or quartenary structure of the FVIII molecule, and to possible exposure of cryptic antigenic sites usually hidden by binding with von Willebrands factor.[22] Mini-epidemics of inhibitors have been ascribed to use of specific batches of plasma-derived FVIII subjected to certain manufacturing processes for purification or viral inactivation.[23,24] However, the apparent increased risk associated with recombinant FVIII is controversial,[25] and possibly due to increased surveillance with identification of previously undetected transient inhibitors.

2. Immunology

The main epitopes recognised by FVIII-inhibitory antibodies lie within the A2, C2 and A3 domains of the FVIII molecule. Multiple antibodies may coexist, with differing and additive functional effects.[26,27] Antibodies to the A2 domain block the FX-activating function of FVIII,[28] anti-C2 antibodies block FVIIIa-phospholipid and possibly FVIII-von Willebrand factor (vWF) binding,[29] and anti-A3 antibodies may interfere with FIX binding.[30]

The immune response to FVIII is T cell-dependent, involving specific T cell receptor (TCR) binding of major histocompatibility complex (MHC)-restricted antigen and B cell synthesis of IgG-4 antibody. The IgG-4 subclass constitutes part of a T_H2 response, mediated by interleukin (IL)-4, IL-5 and IL-10, which promote IgG isotype switch and augment the immune response.[31]

Imbalance of T_H1/T_H2 activity may underlie aberrant immune responses, including autoimmune disease.[32] Although the precise mechanisms involved in the development of inhibitors are not known, it is thought that specific modulation of T cell responses to delete or suppress antigen-specific T cells promoting antibody production

1 Kogenate®; Recombinate®. Use of a trade name is for product identification purposes only, and does not imply endorsement.

may achieve immune tolerance in patients with inhibitors.[33,34]

3. Recognising an Inhibitor

Clinical events may arouse suspicion of inhibitor development, or inhibitors may initially be clinically silent. Inhibitory antibody is routinely detected by an inhibitor screen for a time-dependent antibody and then quantified by Bethesda assay. One Bethesda unit (BU) is the amount of inhibitor which inactivates 50% of 1 unit of added FVIII in 2 hours at 37°C.[35] Immunoassays including immunoblotting[26] and radio-immunoprecipitation[21] demonstrate FVIII-binding antibodies which may shorten the FVIII elimination half-life ($t_{½}\beta$) by immune complex formation and clearance.[21,36] Pharmacokinetic studies measure FVIII recovery and $t_{½}\beta$, and both may be reduced despite absence of FVIII neutralising activity detectable by Bethesda assay.

Patients with FVIII inhibitors are arbitrarily defined according to the maximum inhibitor titre; less than 10BU and greater than 10BU corresponding to 'low' and 'high' responders, respectively. High responders usually demonstrate a strong anamnestic response following repeated challenge with FVIII. Transient inhibitors which persist for less than 3 months with 'on demand' treatment[7] are typically of low titre and do not require ITI.

Since most inhibitors develop during the first early exposures to FVIII, patients at high risk are young PUPs commencing prophylaxis.[37] Surveillance for inhibitors is recommended at 3-monthly intervals or following treatment up to the age of 10 years and yearly thereafter. Inhibitor screening is also undertaken preoperatively, following change of replacement product or failure to respond to previously effective dosages of FVIII.[38] However, this strategy does not include routine testing for noninhibitory antibodies. At our institution, patients who have previously had inhibitors and patients on prophylactic replacement therapy undergo yearly FVIII half-life and recovery studies, which also assist in developing optimal administration regimens based on objective pharmacokinetic data. If an inhibitor is detected, then FVIII replacement should cease immediately pending full evaluation and the development of an individualised management plan. Standard practice in our institution is to defer the start of ITI until the inhibitor titre has reached a nadir, preferably less than 5 BU/ml.[21]

4. Haemostatic Support for Patients with Inhibitors

Optimal management should ideally be supervised by a comprehensive care haemophilia centre with the infrastructure and expertise to monitor and treat this complication.[39]

Arresting significant haemorrhage in the presence of an inhibitor may be problematic and conventional prophylaxis is impossible. In low responders, high dose FVIII on demand may overcome inhibitory antibody by antigen excess. However, anamnestic responses may compromise the usefulness of this strategy. Porcine FVIII has been successful in a limited group of patients with inhibitors which do not cross react, as prophylaxis,[40] for induction of immune tolerance[41,42] and for on demand therapy.[40,43] However, restricted availability, concerns over possible porcine virus transmission and induction of anti-porcine FVIII antibodies have tempered enthusiasm for this approach.

Currently, activated Prothrombin Complex Concentrates (aPCCs) and recombinant activated factor VII (rVIIa) (e.g. eptacog alfa) which bypass FVIII inhibition are the agents of choice for on demand therapy. However, anamnestic responses to residual FVIII and FIX in aPCCs[44] and anaphylaxis to residual FIX in patients with haemophilia B have been reported.[45] These alternative haemostatic modalities are all less effective and predictable in achieving haemostasis compared with FVIII or FIX replacement in the absence of an inhibitor and, therefore, ITI is currently regarded as optimal therapy, allowing effective reintroduction of replacement factor at standard doses.

5. Immune Tolerance Induction (ITI) Therapy

The serendipitous discovery of the phenomenon of immune tolerance to FVIII by Brackmann and Gormsen in 1974[46] led to development of various protocols entailing continuous exposure to FVIII or FIX. Exposure to antigen at either high or low dose may induce tolerance by different mechanisms. Experimentally, high dose antigen induces apoptosis or anergy of antigen-specific T cells, whereas low dose antigen induces antigen-specific regulatory CD4+ T_H2 cells which suppress antibody production nonspecifically.[34,47,48]

ITI protocols are designed to extinguish the inhibitor and ideally restore normal replacement FVIII kinetics. Immunosuppressive and immunomodulatory modalities have been integrated into some protocols. Intravenous immunoglobulin exerts an immunomodulatory effect, possibly by an anti-idiotype mechanism,[49,50] negative feedback on immunoglobulin production and also replaces physiological immunoglobulins removed by immunoadsorption. Direct immunosuppression may be achieved with cyclophosphamide; corticosteroids have not been demonstrated to yield any additional benefit to cyclophosphamide in patients with congenital haemophilia.[51]

Objective assessment of the response to individual regimens entails measurement of values including the inhibitor titre (Bethesda), FVIII recovery, $t_{½\beta}$ and abolition of the anamnestic response to a FVIII challenge.[52] Specific criteria may vary between centres, but success should be defined practically as a negative Bethesda assay and normal FVIII $t_{½\beta}$ that permits standard administration schedules.

It is generally recognised that high dose protocols are more successful, particularly in high responders, and overall success correlates with FVIII dosage.[53,54] Although initial high titres of inhibitory antibody may be reduced by adsorption using staphylococcal protein A columns in an extracorporeal circuit,[55] even in children as small as 17kg,[56] the ability of this technique to improve ITI outcome independently of immunomodulatory therapy has not been properly assessed.

Generally, young patients, low responders and patients with low inhibitor titres before commencement of ITI have the highest success rates.[53] Utilisation of high doses of FVIII and absence of interruptions in therapy also improve success; chronicity of the inhibitor does not appear to affect response.[37]

International registry data demonstrates that tolerance is long lasting; only 1 of 107 patients has relapsed after 4 years, and the median inhibitor-free duration was 5 years (1 to 16.4 years).[53]

5.1 High Dose Regimens

The 'Bonn' protocol devised by Brackmann et al.[57] employs high dose FVIII to induce tolerance (table I). Once normal FVIII recovery and $t_{½\beta}$ is achieved, prophylactic schedules are commenced, but some patients revert to on demand therapy (1999, Brackmann H, personal communication). A recent update presented data from 22 high responders; 7 months mean duration of therapy was required to attain an inhibitor titre of <1 BU/ml and normalisation of pharmacokinetic values, and a mean of 15 months to complete the procedure. Mean FVIII and aPCC utilisation (for haemostatic support) was 2.6×10^6 and 1.8×10^5 units per patient, respectively.[57]

The Malmö treatment model,[55] a high dose regimen with immunomodulatory and immunosuppressive components initially described in 1988 (see table II), has also been successful in ITI of FIX

Table I. 'Bonn Protocol': high dose immune tolerance induction regimen[57]

FVIII 100-150 IU/kg twice daily until inhibitor is undetectable; recovery and modified $t_{½\beta}$ normal, on 3 occasions over a 6- to 8-week period
Dose reduction by 10% every 2-4 weeks
Final tolerance evaluation: washout period of 2-3 days, then assessment of recovery and $t_{½\beta}$ maintained with prophylaxis or on demand treatment
(Activated prothrombin complex concentrates used if haemostatic support is required)

FVIII = factor VIII.

Table II. Malmö treatment model: high dose immunomodulatory immune tolerance induction regimen[55]

Treatment	Time
Extracorporeal immune adsorption	Days 1 and 2
Cyclophosphamide 12-15 mg/kg IV	Days 1 and 2
Cyclophosphamide 2-3 mg/kg po once daily	Starting day 3, for 8-10 days
FVIII to achieve initial FVIIIc of 40-100 U/dl, then 8- to 12-hourly to maintain FVIIIc at 30-80 U/dl	Day 3 onwards, until inhibitor undetectable
IV Ig 0.4g kg/day	Days 4-8
FVIII 30 U/kg/day 2-3 days/week	When inhibitor undetectable

FVIII = factor VIII; **FVIIIc** = FVIII coagulant activity; **Ig** = immunoglobulin; **IV** = intravenous; **po** = orally.

inhibitors. Up until 1996, 20 adult and paediatric patients received treatment (13 haemophilia A and 7 haemophilia B), with an overall success rate of 80%. The mean time to success was 28 days; this rapid response was attributed to minimisation of both inhibitor synthetic rate and concentration at the outset, and maintenance of high FVIII/FIX levels throughout therapy.[58] The use of prednisolone in place of cyclophosphamide has not been as effective (1999, Berntorp E, personal communication).

We recently reported successful results of a 3-phase ITI regimen using recombinant FVIII in 11 children (median age 2 years) with severe haemophilia A and high titre inhibitors (see table III).[21] The median duration of phases 1 and 2 were 6 (range 4 to 12) and 14 (range 8 to 120) weeks, respectively. Phase 2 commenced when neutralising antibody was undetectable by Bethesda assay. Phase 3 commenced when FVIII $t_{½\beta}$ of greater than 5 to 6 hours was documented, and essentially represented adoption of a 'low dose' regimen. The Haemophilia Reference Centre, London and others[36] have shown convincingly that immune tolerance continues to evolve during phase 3, with improvements in $t_{½\beta}$. Phase 3 may also be viewed as a resumption of prophylactic therapy to prevent spontaneous haemorrhage. In adults, a similar dose reducing protocol has been utilised, but with extracorporeal immunoadsorption and intravenous immunoglobulins at commencement.

There is no doubt that ITI therapy is expensive, particularly when high FVIII dosages are used, and there has been historical concern about transfusion of large quantities of human protein in intermediate purity concentrates which may exert an immunomodulatory effect.[59,60] In response to this, several groups have used intermediate or low dose FVIII regimens to achieve immunological tolerance.

5.2 Low Dose Regimens

The Van Creveld model[61,62] uses a low dose schedule of 25 U/kg FVIII on alternate days, with reduction when the FVIII recovery exceeds 30%, until prophylactic doses of 10 to 15 U/kg 3 times a week are attained. The overall success rate was approximately 87%, and 100% in patients with an inhibitor titre less than 40BU compared with 75% in patients with a titre greater than 40BU. Tolerance was attained within a median of 1 year (range 0.5 to 28 months); 6 and 19 months for patients with maximum titres of less than 40BU and greater than 40BU, respectively. The duration of treatment was directly proportional to the maximum Bethesda titre and inversely proportional to the age at inhibitor development.[61]

5.3 Factor IX Inhibitors

Although ITI schedules may be effective for FIX inhibitors, experience with them is limited. There have been recent reports of nephrotic syndrome developing after therapy in patients with high-titre inhibitors, which has thus far not been reported in patients with haemophilia A.[2,63] Aller-

Table III. Three-phase progressive dose-reducing immune tolerance induction regimen[21]

Phase 1: FVIII 200 IU/kg/day
Phase 2: FVIII 100 IU/kg/day, when Bethesda[a] < 1 BU/ml
Phase 3: prophylactic FVIII 50 IU/kg 3 times per week, when FVIII elimination $t_{½\beta}$ > 5-6 hours

Bethesda = assay to quantify inhibitory antibody - 1 Bethesda unit (BU) is the amount of inhibitor that inactivates 50% of 1 unit of added FVIII in 2h at 37°C; **FVIII** = factor VIII.

gic reactions to both FIX[44] and FVIII[64] have been reported.

5.4 Supportive Measures During ITI

Achieving effective haemostasis in patients with inhibitors who haemorrhage during ITI may be an added challenge. In general, management involves the use of either aPCCs or rVIIa,[65] as in patients who are not undergoing ITI. Venous access may prove difficult in any ITI regimen, and fully implanted central venous access devices should be considered. In addition, high flow rate dual lumen dialysis catheters may be required for venous access during extracorporeal immunoadsorption. In our experience, both of these may be inserted safely with appropriate haemostatic support.

5.5 Failure of ITI

Failure is difficult to define, with published studies utilising different criteria for success. Furthermore, the duration of ITI on high and low dose regimens will differ. In general an ITI regimen could be deemed to have failed based on objective investigations after 18 to 24 months of uninterrupted therapy. However, subjective improvements in haemorrhagic symptoms despite subnormal recovery or $t_{½\beta}$ should be considered in assessing 'failure'.

5.6 Economics

Few studies address the economics of inhibitor management in patients with haemophilia and long term (>5 years), prospective, case-controlled studies are required to resolve ITI versus supportive treatment cost-benefit analysis.[66] The potential long term benefits of success may justify high short and medium term costs, and each case has to be assessed individually. It is estimated that for patients who have not been tolerised, high responder patients the cost of hospital treatment and factor replacement differs 10-fold from the low responder or patients without inhibitors.[67] Apart from quantifiable financial costs of factor usage and hospital admission, the quality of life, orthopaedic outcomes, analgesic dependency and psychosocial adaptation warrant consideration in comparative costing of radical or conservative strategies in patients with inhibitors. Undertaking ITI requires a high degree of commitment from the patient and family, and compliance has an influence on outcome.

6. The Future

It has been more than 25 years since ITI was initially performed, and there are still numerous unanswered questions. Evidently different approaches are effective in eradicating inhibitors and the optimal ITI programme has yet to be defined. ITI has thus far not been amenable to rigorously controlled, prospective, randomised clinical trials because of the relatively small numbers of patients involved. ITI should be centralised nationally or regionally to allow for the accumulation of experience and expertise in the setting of formal prospective studies. The role, if any, of alternative immunosuppressive agents such as cyclosporin and tacrolimus has not been formally assessed. Modulation of immunoglobulin production via CD40 through its ligand CD40L[68] is an exciting possibility. Evolving recombinant technology has facilitated the synthesis of engineered FVIII or FIX molecules with substitution of porcine sequences at key human FVIII antigenic sites.[69] Another theoretical approach involves the use of key peptides from the FVIII molecule to induce tolerance in high risk patients.[32]

Although rare, the impact of inhibitors is phenomenal, with major repercussions both for the patients and for the funding of their management. It is therefore essential to determine the optimal approach to the assessment and therapy of this iatrogenic complication.

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