Von Willebrand factor-containing factor VIII concentrates and inhibitors in haemophilia A

A critical literature review

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Summary
The development of inhibitors that neutralise the function of factor VIII (FVIII) is currently not only the most challenging complication associated with the treatment of haemophilia A but it also increases the disease-related morbidity as bleeding episodes do not respond to standard therapy. The main short-term goal of the treatment of inhibitor patients is to control bleeding episodes while the long-term one is to permanently eradicate the inhibitor by immune tolerance induction, particularly in the case of high-titer antibodies. Due to some in vitro studies and clinical observations, some investigators have suggested that FVIII concentrates containing von Willebrand factor (VWF) may be less immunogenic than high-purity or recombinant FVIII products. It has also been suggested that success rates for immune tolerance induction are higher when plasma-derived FVIII products are used. The currently available data from laboratory and clinical studies on the role of VWF in inhibitor development and eradication in haemophilia A is critically analysed in this review. As a result, we have not found definitive evidence supporting a role for product type on inhibitor incidence and inhibitor eradication in haemophilia A patients.

Keywords
FVIII, inhibitors, haemophilia A, immune tolerance induction (ITI), VWF

Introduction
The development of inhibitory antibodies against factor VIII (FVIII) is the most serious complication of replacement therapy in congenital haemophilia A (haemA) (1), occurring in approximately 15–30% of severely affected previously untreated patients (PUPs) and resulting in the lack of response to therapeutic FVIII (2–7).

A number of prognostic factors such as genetic, environmental and treatment-related factors have been proposed to affect inhibitor formation (8). With respect to genetic risk factors, inhibitors are especially frequent in patients with underlying mutations that prevent formation of FVIII protein, such as deletions of large portions of the gene, nonsense mutations causing premature stop codons and inversion of the FVIII gene (9–11). In addition, other genetic factors have been proposed to influence inhibitor formation, such as polymorphisms in the FVIII gene encoding for different wild-type FVIII proteins or in genes involved in the immune response, such as the major histocompatibility complex (MHC) and cytokines (e.g. IL-10, TNF-α, CTLA-4) (12–15). The role of environmental or treatment-related factors, such as age at first factor concentrate exposure, intensive FVIII exposure, immunologic challenge or type of FVIII replacement therapy (i.e. recombinant or plasma-derived) in the likelihood of developing inhibitors has been explored by a number of studies with conflicting results (16–18). Even less understood is the inter-relationship between different risk factors for the development of inhibitors. For instance, Viel et al. showed preliminary evidence that mismatched FVIII replacement therapy to an individual patient’s wild-type FVIII protein (designated H1 through H6) may be a risk factor for inhibitor development (12). The risk factors in regards to treatment modality and immunologic challenge are also connected as demonstrated by Kurnik et al. The authors developed an early prophylaxis regimen that reduces the incidence of inhibitor development by inducing tolerance in the absence of immunologic danger signals (19). It is therefore clear that inhibitor formation in haemophilia is a complex multifactorial process.

In the late 1980s, regulatory authorities identified PUPs as the appropriate population for FVIII pre-licensure trials for virus safety testing. The trials investigating new rFVIII products subjected patients to frequent inhibitor testing. The initial cumulative incidence of inhibitors from these trials was 25–32%, approximately two-fold higher than expected (historic inhibitor prevalence with plasma-derived FVIII [pdFVIII] ranged from 0–12%).
leading some to speculate that rFVIII products were associated with a higher rate of inhibitor formation than pdFVIII (4, 20, 21). However, it was unclear if the rate of inhibitor formation associated with rFVIII products partially reflected the natural history of inhibitors in a highly selected and carefully monitored patient population (22). In contrast, many of the pdFVIII trials studied small patient populations, were retrospective, and included multiple types of pdFVIII products with varying characteristics. Given the differing study designs and patient populations, comparisons between pdFVIII and rFVIII trials are not scientifically sound. Inhibitor detection remains partially dependent on methodology with a large inter-assay and intra-assay variability (23, 24). Subsequently different results can be obtained using the same samples in different laboratories using different methodologies (e.g. Nijmegen or standard Bethesda assay for inhibitor titer and chromogenic or one-stage assay for FVIII activity) (25). Furthermore, as the influence of a single risk factor, such as factor type, on inhibitor formation is difficult to separate from genetic and environmental factors, the Factor VIII and Factor IX Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH) put forth recommendations concerning patient populations appropriate for evaluation of new FVIII concentrates (26). The committee stated that as PUPs and non-infected patients have a certain but undefined likelihood of inhibitor formation, they may be less useful in evaluating the immunogenicity of new factor concentrates. Further, previously treated patients (PTPs) with greater than 150 exposure days (EDs) are generally considered tolerant of factor replacement therapy and thus the committee recommended that immunogenicity of new concentrates be analysed in PTPs with greater than 150 EDs (26).

The main short-term goal of the treatment of inhibitor patients is to control bleeding episodes, while the long-term one is to permanently eradicate the inhibitor (1). Immune tolerance induction (ITI) towards exogenous FVIII represents the only therapeutic approach effective for eradication of FVIII antibody and restoration of normal FVIII pharmacokinetics (27). Current literature reports success rates ranging between 60–80%, depending on the protocol utilised (28–35). One hotly debated issue in ITI therapy is whether factor type impacts outcomes. Some have hypothesised that ITI using pdFVIII containing VWF (FVIII/VWF) might result in greater success (36–40). However, the role of VWF in FVIII products used for the purposes of ITI, if any, has not been fully elucidated.

The aim of this review is to analyse current literature on the potential role of FVIII/VWF in inhibitor development and eradication in haemA patients. Currently discussed hypotheses on possible mechanisms of the role of VWF in FVIII immunogenicity are described along with associated preclinical studies. Clinical trials determining inhibitor incidence of FVIII products are reviewed as well as clinical trials evaluating ITI success rates.

The postulated protective role of VWF: hypothetical mechanisms

Von Willebrand factor is a large glycoprotein with a crucial role in haemostasis, initiating platelet adhesion at sites of vascular injury (41). In addition, VWF transports FVIII to sites where it can participate in forming fibrin clots, prolongs the half-life of circulating FVIII, protects FVIII from proteolytic inactivation and increases FVIII concentration within the forming haemostatic plug (41). The controversy lies in whether FVIII/VWF, namely low- to intermediate-purity pdFVIII carry an advantage over products that do not contain VWF such as high-purity or monoclonal pdFVIII and rFVIII products.

Epitope masking

There are two main mechanisms hypothesised to explain a protective role against inhibitor development of FVIII/VWF: epitope masking of FVIII to reduce immunogenicity and protection from FVIII endocytosis by dendritic cells (DCs). The first hypothesis is based on the fact that binding sites for VWF on the FVIII molecule (Fig. 1) are also inhibitor epitope sites (the amino terminal region of the light chain corresponding to the A3 domain and the carboxy terminus within the C2 domain) (42–44). As a result, VWF contained in pdFVIII may protect and mask these epitopes, thus preventing FVIII inhibitor binding. Epitope masking may account for the observation in some in vitro studies that FVIII/VWF appears less immunoreactive than high-purity pdFVIII (45–51) and generates more thrombin (51).

The variation in inhibitor immunoreactivity was evaluated in vitro using four commercial FVIII products with varying amounts of VWF (44): Fanhdi (Grifols, Cambridge, UK), Haemate-P (CSL Behring, West Sussex, UK), Hemoct-M (Baxter Healthcare, Deerfield, IL, USA), and Kogenate® Bayer (Bayer Healthcare, Pittsburgh, PA, USA) in the presence of FVIII-inhibitor containing plasma of varying titers. From this experiment the authors concluded that FVIII products high in VWF (Fahndi, 1:1 ratio of VWF:FVIII and Haemate 2.5: 1 ratio) were less sensitive to al-

![Figure 1: VWF binding sites on the FVIII molecule.](image-url)
loantibody inhibition than products lower in VWF (Hemofil-M and Kogenate® Bayer) based on the former’s lower inhibitor titers in the Malmo assay and greater inhibitor resistance in the thrombin generation assay (TGA).

However, as described by Verbruggen et al., it is important to consider that varying levels of VWF; dilution of TGA samples, and clinically irrelevant experimental conditions are methodological limitations of this study (52). The greatest caveat is that the study was performed in mixtures with varying VWF because of the different dilution rates of the patient plasma samples in conjunction with the use of VWF-free diluent. In standard inhibitor assays performed by the authors and in contrast to the Salvagno et al. study, low-VWF pdFVIII concentrates typically have higher inhibitor titers than both high-VWF pdFVIII and rFVIII, which has no VWF. This suggests a lack of correlation between VWF content and inhibitor titer. The TGA is dependent upon VWF from FVIII preparations; therefore, VWF-free FVIII deficient plasma as a diluent deprives the TGA of the uniformly protective effect of VWF from inhibitors, particularly those samples using rFVIII (53–55). This dilution artifact explains why the most-diluted samples have the highest inhibitor titer variations when tested against varying FVIII concentrations. The fact that no VWF-related titer differences were noted between rFVIII and pdFVIII in Bethesda inhibitor assays supports the hypothesis that assay-related dilution artifacts account for inhibitor titer differences between rFVIII and FVIII/VWF in the TGA. VWF is not a limiting factor in vivo in haemA patients; as FVIII preparations are administered, both rFVIII and pdFVIII are protected from inhibitor antibodies to the same degree. As noted by Verbruggen et al., the use of in vitro methods to develop algorithms predictive of epitope-specific in vivo effectiveness of FVIII concentrates presents a challenge (52). In Verbruggen’s opinion, the inhibitor assay and TGA should mimic clinical situations as closely as possible, particularly with respect to VWF concentration and protein content of diluted samples (52). Therefore, the data reported by Salvagno et al. do not appear to support their conclusion that varying VWF concentrations in FVIII products are relevant in in vitro or in vivo for the inhibition of FVIII.

Suzuki et al. studied the in vitro role of VWF in regulating inactivation of FVIII by C2-domain-specific inhibitors by comparing the kinetics of inactivation of rFVIII (Kogenate; no longer marketed) and FVIII/VWF (Haemate P) (47). Inhibitor titers for the two FVIII products showed discordance depending upon epitope specificity, namely anti-C2 antibodies were less inhibitory to FVIII/VWF. For 10 inhibitors with C2-domain reactivity, dose-dependent inhibition of the FVIII-VWF complex formation was observed, whereas for A2-domain specific inhibitors, there was no inhibition of complex formation. The authors concluded that VWF-bound FVIII is less accessible to the FVIII light chain-specific antibody and exhibits a lower inactivation rate. The results of this study suggest that inhibitor titers can differ according to the amount of VWF in the reference source of FVIII. However, this conclusion is not relevant in a clinical setting as the in vivo situation is quite different from the assay conditions. In haemA patients, therapeutic FVIII is administered via i.v. into blood containing a 50-fold excess of VWF. Due to the low dissociation constant (Kd) of FVIII towards VWF (Kd=0.4 nM), the FVIII-VWF complex assembles within 12 seconds (56). The average Kd of affinity-purified natural anti-FVIII antibodies toward FVIII is 3.8 ± 1.81 nM (57) which is about 10-fold higher than the Kd for FVIII and VWF (56). Theoretically, if a patient had a rare anti-C2 antibody with a lower Kd for binding to FVIII than VWF, then these findings may have some clinical significance. However, thorough clinical studies and kinetic analysis of patient inhibitor plasma would be necessary to confirm this finding. Moreover, the half-life of FVIII and recovery is the same in patients regardless of treatment with either FVIII/VWF or full-length rFVIII products (58). If pre-complexed FVIII/VWF conferred superior stability in a clinical setting, the FVIII half-life should be longer compared with that of rFVIII.

To evaluate whether epitope profile affects the neutralization of pdFVIII and rFVIII in vitro, the plasma samples from patients with severe haemA and high-responding inhibitors were evaluated using two pdFVIII products (Haemate [high-VWF] and Monoclate-P (CSL Behring) [low-VWF]) and three rFVIII products (Helixate [CSL Behring], Recombinate [Baxter Healthcare], and ReFacto [Wyeth, Madison, NJ, USA]) (49). Immunoreactivity against Haemate was lower than against Monoclate-P in 10/24 samples, comparable in 13/24 samples and higher in only one case. When Haemate titers were measured against Helixate, a rFVIII product which does not contain VWF, only 6/24 patients demonstrated lower titers for Haemate, 11/24 patients had the same titer and in 7/24 patients, the titer is higher with Haemate. Interestingly, in most cases where the titer with Haemate is lower than with Monoclate, it is higher for Haemate when compared with Helixate. Since neither Monoclate nor Helixate contain significant concentrations of VWF, it is difficult to claim a correlation between VWF presence and titer differences in the samples. Furthermore, the authors used a chromogenic assay to identify FVIII:C and Malmo units to measure inhibitor titer. Most laboratories use a Nijmegen or standard Bethesda assay with a one-stage clot based assay to identify inhibitor titer. The large inter-assay variability in inhibitor detection could affect the results and thus the conclusions of these in vitro tests. The authors opined that based on their findings, the differences in FVIII concentrates could allow optimisation of bleeding management and ITI; however, the results do not support that high-VWF content in pdFVIII confers a treatment advantage to patients with inhibitors.

Epitope masking of FVIII by VWF would theoretically occur predominantly at the C2 or A3 domain of FVIII. However, Prescott et al. studied domain specificity in inhibitor patient groups treated only with pdFVIII or rFVIII and saw no difference in the percentage of inhibitor patient plasma binding to the C2 domain (83% pdFVIII, 81% rFVIII). Antibodies to AR3-A3-C1 were present in 61% of patients treated with pdFVIII but only 18% of rFVIII treated inhibitor patients. The authors conclude that FVIII inhibitors are complex and heterogeneous and there is no clear difference in reactivity caused by pdFVIII or rFVIII administration (59).

Lin et al. hypothesised that FVIII protein content (FVIII:Ag) per unit of FVIII:C in FVIII products in addition to efficiency of FVIII:Ag binding to VWF may provide relevant information to
predict inhibitor risk and direct therapeutic selection (60). The FVIII:Ag content of the products (rFVIII manufactured by Bayer or Baxter, pdFVIII manufactured by Aventis Behring, Baxter, Cutter/Miles, Grifols) was measured and binding to VWF was determined by Size Exclusion (SE)-chromatography. The rFVIII preparations contained significantly more FVIII:Ag per IU of FVIII than the pdFVIII concentrates (p < 0.05). Approximately 20% of the rFVIII:Ag did not complex with VWF. The authors concluded that unbound FVIII is denatured or damaged and could cause increased immunogenicity. However, activity assays were not performed to determine the amount of active FVIII compared with total protein. Instead, the authors used the nominal activity provided by the manufacturers. As different manufacturers use different assays, buffer conditions and standards to measure the activity of their FVIII products, it is not possible to directly compare the activity data. In addition, during the manufacturing process different methods (e.g. pasteurisation) are implemented and may lead to inactivated FVIII molecules. Therefore even in a single FVIII product the FVIII antigen content may vary from batch to batch although the FVIII:C activity is comparable. Furthermore, the omission of activity assays in the study undermines the SE-chromatography results as it is unclear whether the chromatography process itself causes FVIII to denature, thereby reducing or eliminating its VWF binding capability. Vlot et al. performed SE-chromatography followed by an activity assay and saw that up to 75% of the FVIII sample is denatured during the chromatography process alone (56). Similar results using a different resin are reported by Koedam et al. (61). This denaturing of FVIII in the assay would prevent FVIII binding to VWF, thereby rendering the results an artifact of the experimental conditions.

Protection from endocytosis by dendritic cells

Regarding the second potential mechanism, in vitro studies indicate that VWF reduces FVIII immunogenicity by preventing FVIII endocytosis by DCs, which are the professional antigen presenting cells (APCs) involved in the primary immune response to exogenous FVIII. Dasgupta et al. demonstrate that pre-incubation of VWF with FVIII reduces the uptake and presentation of FVIII by DCs compared with FVIII alone (62, 63). However, the absence of VWF alone is not sufficient to render FVIII more immunogenic. The plasma of non-haemophilia individuals contains a dynamic equilibrium of 2–8% free FVIII (64). Once dissociated from VWF, FVIII is rapidly cleared and removal of free FVIII is compensated with the release of more FVIII from VWF. In haemophilia individuals, the absolute concentration of free FVIII would be lower than non-haemophiliaics since treatment does not aim for 100% replacement of FVIII levels. Also, both immune response and immune tolerance require separation of FVIII from VWF and uptake of FVIII by APCs. Interestingly, Pfistershammer et al. demonstrated that neither FVIII, thrombin-activated FVIII, VWF nor a complex of FVIII and VWF induces the maturation of human DCs, the stimulation of allogeneic or autologous T cells or modulation of the release of cytokines by human DCs (65). The authors conclude that FVIII, whether alone or complexed with VWF, does not possess inherent danger signals for human DCs and therefore would not stimulate an immunogenic response. On the contrary, the absence of danger signals should result in immune tolerance (66). Changes in the actual FVIII molecule are required at the sequence or structure level to render it immunogenic, the therapeutic relevance of which can only be determined through clinical studies.

Clinical relevance of FVIII/VWF preparations in inhibitor development

The hypothesis that VWF may have protective capabilities against inhibitor development arose through anecdotal clinical observation. In the pivotal trials conducted for rFVIII product approval, inhibitor incidences in PUPs with severe haemA ranged from 15% to 32% (summarised in Table 1) Historic inhibitor rates for pdFVIII products were considered lower mainly based on retrospective studies. Available prospective PUP studies on pdFVIII demonstrate an inhibitor rate between 3–52% for severe (FVIII<1%) haemophilia A patients (Table 1) (6, 67, 68). Ehrenforth et al. reported inhibitor development in 24% (15/63) of all PUPs and 52% (14/27) of severe PUPs treated with pdFVIII (67). Of the 15 patients who developed inhibitors, 13 had been treated with an intermediate-purity FVIII concentrate. The findings indicate that previous reports of pdFVIII therapy might have underestimated the risk of developing FVIII inhibitors. However, it is difficult to draw any comparative conclusions based on data from studies performed with different designs, testing frequency, patient populations and time periods.

In a prospective, comparative study of PUPs with moderate to severe haemA, Kreuz et al. detected inhibitors in 18/51 (35%) patients treated with pdFVIII and 4/21 (19%) patients treated with rFVIII (69). In the severe subgroup, inhibitors developed in 46% of pdFVIII and 36% of rFVIII treated patients. No difference was observed in the development of high-titer inhibitors among the severe group treated with pdFVIII (13/35; 37%) compared with rFVIII (4/11; 36%).

Studies for most rFVIII product approvals were required in PUPs prior to the ISTH recommendation that immunogenicity of new concentrates be analysed in PTPs with greater than 150 EDs (26). Pivotal studies in the PTP population with severe haemA demonstrated an inhibitor incidence of <1% for Recfacto and 0% for Recombinate, Kogenate and Kogenate FS/Bayer (70–74). PTP prospective trials for Advate resulted in inhibitor rates of 0–<1% (75). In examining the most appropriate patient population for determining immunogenicity, the rFVIII products are no more clinically immunogenic than pdFVIII products, which show rates between 0–<1% (76–78) (Table 2).

Several reviews have comparatively analysed the incidence of inhibitor development in haemA patients treated with FVIII/VWF or rFVIII products. In 2003, Wight and Paisley published a system-
atic review investigating the association of FVIII product type with inhibitor formation (4). A total of 50 relevant retrospective or prospective studies were identified in the literature. A comparison of 13 studies on PUPs found that patients treated with pdFVIII had a lower cumulative inhibitor incidence than those treated with rFVIII (12, 16, 17). In patients treated with pdFVIII, the cumulative inhibitor incidence ranged from 0–12.4% (weighted mean: 6.8%) for all inhibitors and from 0–2.5% (weighted mean: 1.4%) for high responders (>5 Bethesda Units, BU). In comparison, patients treated with rFVIII reported cumulative inhibitor rates between 36–38.7% (weighted mean: 37.5%) for all patients; for high responders, the inhibitor incidence ranged from 11.3–18% (weighted mean: 15.1%). However, several methodological criticisms have been raised against this review which compared very heterogeneous trials in terms of study design (e.g. prospective/retrospective, frequency and method of inhibitor testing) and study populations (ethnicity, type of gene mutation, age at first exposure to FVIII, etc.) (40, 79), making it impossible to draw any conclusion based on the comparison of inhibitor incidence of the different products across studies. In addition, it seems incorrect to pool all pdFVIII and all rFVIII products as they greatly differ from each other. Indeed, the VWF amount varies among commercial pdFVIII and important differences also exist among rFVIII products (e.g. antigen content). The limitations above also apply to recent analyses through literature reviews presented in abstracts by Iorio et al. (80) and Halimeh et al. (81) comparing rates of inhibitors of haemA patients treated with pdFVIII or rFVIII. Halimeh et al. conducted a meta-analysis of five cohort studies resulting in 916 haemA patients and saw a statistically significant association with inhibitor development for rFVIII-treated patients (odds ratio/95% confidence interval [CI] of 1.8/1.2–2.6 for high responders). Iorio et al. reported 20 trials in PUPs with mild, moderate or severe haemA from 1990–2001. Of the severe patient population, 80/643 (12.4%) patients treated with pdFVIII and 94/510 (18.4%) of rFVIII-treated patients developed inhibitors. However, the review pooled prospective and retrospective trials, resulting in a risk of double-reporting. As these studies were presented in abstracts, it is difficult to analyse their methodology and results. For example, it was unclear whether FVIII gene mutations were controlled for in the analyses (80).

Data from a retrospective evaluation of 148 severe PUPs have been published relatively recently by Goudemand et al. (82). Sixty-two patients were treated exclusively with pdFVIII concentrates and 86 with rFVIII products. According to a Cox multivariate analysis excluding other possible risk factors (FVIII gene mutation, ethnicity, family history of inhibitor, age at first infusion), rFVIII was found as an independent risk factor for inhibitor development and carried an approximately 2.4- to 3.2-fold higher risk than FVIII/VWF. When restricting the analysis to high responders, no statistical significance was found for this cohort (p = 0.157), whereas the results remained significant in patients with high-titre inhibitors and/or immune tolerance induction (patients who were started on ITI directly after inhibitor detection) (p = 0.045). Similarly, Chalmers et al. reported in a retrospective study on 348 severe

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<th>FVIII Products</th>
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<th>Study type &amp; duration</th>
<th>Severity (n)</th>
<th>Definition of severity (% FVIII)</th>
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*37 PUPs, 24 MTPs.
haemA children found that inhibitors developed more frequently in patients initially treated with rFVIII when compared with pdFVIII (27% vs. 14%, respectively; p = 0.009) (83). However, a large retrospective analysis of data on 316 PUPs enrolled in the CANAL (Concerted Action on Neutralizing Antibodies in severe haemA) cohort study contradicted the conclusions by Goude-mand et al. and Chalmers et al. (84) Indeed, the authors found that pdFVIII with considerable quantities of VWF carried the same inhibitor risk than rFVIII products (relative risk [RR], 1.0; CI 0.6–1.6). Also switching between FVIII products did not increase the risk of inhibitors (RR 1.1; CI, 0.6–1.8).

Overall, the contradictory results of these retrospective studies confirm the lack of convincing data on a protective role of VWF in inhibitor development and there is a need for prospective, randomised, well-designed comparative trials. Two prospective, comparative studies have been launched, the first conducted by the German, Austrian, and Swiss Society of Thrombosis and Hemostasis Research (GTH) PUP Study Group (85). A total of 324 haemA or B PUPs have been recruited since 1993. Preliminary analysis of 104 severe haemA PUPs exposed at least once to rFVIII or pdFVIII revealed a small, but non-statistical difference (21% vs. 36%; p = 0.08) in inhibitor development. The second multicentre trial, the Study on Inhibitors in Plasma Product-Exposed Toddlers (SIP-PET), a prospective, open-label, randomised, controlled trial of inhibitor development in PUPs exposed to FVIII/VWF and rFVIII concentrates, is expected to start in the near future (86).

### The role of VWF containing FVIII concentrates in inhibitor eradication

The choice of therapeutic products is one of the most controversial issues in the current debate on ITI (87, 88). Some investigators have observed enhanced efficacy of FVIII/VWF in achieving tolerisation. A longitudinal study conducted by Kreuz et al. showed that success rates using a high-dose ITI protocol (the Bonn protocol) declined from 91% (19/21) to 37.5% (6/16) with the introduction of high-purity concentrates (85). Furthermore, when patients who had an unsatisfactory response to ITI using monoclonal pdFVIII or rFVIII were switched to FVIII/VWF, 80% (8/10) were tolerised. It is important to note that patients were only on the high-purity...
products for a median duration of three months whereas when switched to FVIII/VWF, ITI was achieved after a median of three months (range 5–36). A similar experience has been reported for patients treated at the haemophilia centres of Bonn and Bremen, Germany (89). Prior to 1990, 51 patients received ITI using exclusively FVIII/VWF and between 1990 and 2001, 42 patients received ITI with either FVIII/VWF or rFVIII products. During the first period the overall success rate was 87%, while during the second period the success rate was lower for those patients treated with rFVIII (54%), but remained similar for patients treated with FVIII/VWF (82%). However, the comparison is inadequate to draw conclusions from as the patients receiving FVIII/VWF or rFVIII likely differed significantly in characteristics that could account for the difference in ITI success rates.

To date, the majority of evidence suggesting a role for FVIII/VWF in ITI success is derived from small in vitro studies (90, 91), small case series (89, 92), retrospective studies (93, 94), and small uncontrolled studies (95). Orsini et al. reported that ITI was successful in 7/8 patients undergoing ITI with a high-purity FVIII/VWF product (the remaining patient relapsed after becoming inhibitor-free but remained on FVIII treatment and was considered a partial success) (92). In addition, a prospective multicenter study conducted by Gringeri et al. on 17 haemophiliacs at high risk for a poor response to ITI, showed that nine patients (53%) obtained complete tolerisation using high-purity FVIII/VWF (95). The remaining seven patients converted from high- to low-responder status. A retrospective chart review of 11 patients receiving FVIII/VWF for ITI indicated that 63% of patients achieved complete or partial tolerisation (93).

More recently, the efficacy of ITI using rFVIII in 26 patients with severe haemA and high-titer inhibitors was evaluated (96). Twenty inhibitor patients received the same rFVIII product that induced the inhibitor. Complete success was achieved by 73% and partial success by 8% of patients. Two patients who partially responded developed catheter-related infections, supporting the theory that intercurrent infections, which may stimulate the immune system (28), may influence ITI success.

A prospective study of ITI in 14 children (13 high responders) with severe haemA at a single institution was conducted (97). Ten (71%) patients received rFVIII and four (29%) received low- or high-purity pdFVIII. Comparing the efficacy of the various factor types was not an endpoint of this study. Overall tolerance was achieved in 79% of patients (57.1% complete, 21.4% partial).

Since rFVIII has been used nearly exclusively in Canada from 1994, a multi-centre, retrospective survey of ITI cases was conducted (36). Of 29 patients completing ITI, 86% received rFVIII exclusively and 14% received pdFVIII exclusively or pdFVIII followed by rFVIII. With a median duration of ITI of 1.1 years (range 9 days – 6 years), the overall success rate of all patients was 79% which is comparable to that reported by immune tolerance registries. There was no significant difference (p =0.55) between pdFVIII and rFVIII products in terms of ITI success (98). The authors concluded that rFVIII is not inferior to pdFVIII for ITI.

The efficacy of Kogenate (no longer marketed) for ITI was retroactively evaluated in 11 patients with severe haemA (7 high responders) from France, Germany, Spain and Switzerland (99). All patients received Kogenate for ITI, although one patient initially received pdFVIII. A total of 82% of patients were successfully tolerised, with 78% of high responders achieving success. Valentino et al. retrospectively analysed 12 ITI patients who underwent treatment using Advate (Baxter Healthcare) (100). Tolerance was achieved in nine patients (75%), including 7/10 (70%) with high-titer inhibitors. The median time to success was four months and as of the time of publication, two patients were still undergoing ITI (52 months and 27 months on treatment) and in one patient ITI had failed after 44 months of treatment.

In a meta-analysis published in 1999 from the International Immune Tolerance Registry (IITR) and the North American Immune Tolerance Registry (NAITR), among the variables analysed (e.g. historical peak inhibitor, inhibitor titer before ITI initiation, FVIII dosing and FVIII products), only a historical peak inhibitor titer <50 BU and an inhibitor titer <10 BU immediately before ITI initiation were identified as predictors of ITI success (101). A 2009 meeting report of the NAITR reiterated that FVIII product purity had no impact on ITI success in the cohort of 164 haemA subjects who completed ITI. The rate of ITI success of subjects tolerised with intermediate- or low-purity products was 67.5% and not statistically different from patients successfully tolerised with monoclonal or rFVIII (71%). The overall success rate of ITI was 70% (115/164), with 63% (81/128) of high responder subjects successfully tolerised (30). In addition, in a retrospective study conducted by Kurf et al. on 25 haemophiliacs with high-titer inhibitors (94), complete tolerisation using various FVIII/VWF occurred at a lower rate (32% complete success) and required a longer period than previously reported (85, 88, 89). Many of the patients who achieved tolerisation switched back to a rFVIII product for prophylaxis and all have maintained tolerance.

In absence of robust evidence from methodologically rigorous randomised trials with adequate sample sizes, at the present time no definitive conclusions can be drawn on the superiority of any FVIII product in ITI. In this context, with the aim to provide useful information to haemophilia caregivers, an international panel of haemophilia opinion leaders developed consensus recommendations for ITI on the basis of available published literature and the collective clinical experience of the group (27). The main consensus recommendations state that ITI is successful using FVIII products regardless of VWF content, with no definitive data supporting the superiority of any FVIII product, and that most patients can be effectively tolerised with the same FVIII product in use at the time of inhibitor detection.

To obtain additional evidence-based data regarding the influence of FVIII product type on ITI success, several prospective trials have been launched. In the prospective, randomized Rescue Immune Tolerance Study (RESIST)-naive, ITI-naive patients with poor prognostic factors will be randomly assigned to FVIII/VWF or rFVIII at a dose of 200 IU/kg daily. In the RESIST-experienced trial, patients who have previously failed ITI with monoclonal or recombinant FVIII will undergo ITI using FVIII/VWF concentrates at a dose of 200 IU/kg day (95). The observational ITI Study, ObsITI, a multicentre study initiated by the Frankfurt haemophi-
lia centre, is also investigating whether the presence of VWF in FVIII concentrate impacts ITI success (39). The recently closed International Immune Tolerance Induction Study compares the efficacy, response time, morbidity and economics of a high-dose and a low-dose immune tolerance protocol. While it is not focused on effect of product type, the study seeks to identify predictors of successful ITI.

Conclusions

There remains no definitive evidence about the impact of FVIII product type on inhibitor incidence and inhibitor eradication in ITI treatment in haemA patients. Some studies supporting an immunoprotective role of VWF draw conclusions of immunogenicity without providing evidence for any clinical significance of differences in immune reactivity seen in their tests. It is important to note that the studies focused on epitope masking of FVIII by VWF do not address inhibitors binding outside the C2 and A3 domains. Inhibitors are polyclonal antibodies and thus can bind across nearly all domains of FVIII. Due to the paucity of conclusive data, there is no obvious correlation between VWF content of FVIII preparations and immune response in patients.

Clinical comparisons of inhibitor development and ITI success rates from different patient populations conducted under various study designs and analytical methods are scientifically unsound. In this context, the results from the ongoing randomised prospective trials are awaited and will help resolve many unanswered issues. However, until these studies are concluded, we think that it is unjustified to modify the current evidence-based guidelines of most haemophilia organisations which recommend that, where available, rFVIII products are the treatment of choice of haemA patients and that FVIII inhibitors must be tolerated with the same agent used at the time of inhibitor detection (27, 102–106).

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References


