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ORIGINAL ARTICLE *Inhibitors and allergic reactions*

Immune tolerance induction in 31 children with haemophilia A: is ITI less successful in African Americans?

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Summary. Inhibitor development continues to be a major problem in the treatment of haemophilia. Immune tolerance induction (ITI) continues to be the most effective approach to managing this complication. This study reviews the practice and outcome of ITI at a single centre over a 17-year period. All 31 inhibitor patients have haemophilia A. Two patients with haemophilia A underwent two trials of ITI and a third patient underwent three trials of ITI for a total of 35 courses of ITI in these 31 patients. Most patients had high responding inhibitors, 22 of 31. Seventy-one percent of haemophilia patients achieved tolerance. Courses of ITI in African American (AA) patients with haemophilia A were much less likely to achieve tolerance compared with non-AAs, 57.9% and 92% (P = 0.04) respectively. Most trials of ITI were carried out with recombinant products (25 of 35). While ITI continues to be an effective therapy for patients with inhibitors, it is less effective in AA patients, and patients with higher inhibitor titres. In this refractory group of patients, new approaches are needed.

Keywords: ethnic differences, factor IX, factor VIII, haemophilia A, immune tolerance, inhibitor

Introduction

Approximately 30% of haemophilia A patients develop inhibitor antibodies to replacement clotting factors [1,2], rendering first line therapies ineffective and requiring the administration of less effective and more costly bypassing agents [3]. Immune tolerance induction (ITI) was first described in the 1970s by professor H.H. Brackmann of Bonn, Germany, involves administration of frequent large doses of factor VIII (FVIII) products along with activated prothrombin complexes (APC) on a regular basis to induce the immune system to stop production of the inhibitor antibodies [4]. This strategy has proven successful in the majority of patients with haemophilia A over the past few decades and ITI with lower dose regimens has also proven effective [5]. The optimal agent, dose and infusion schedule for each patient have not been determined and many controversies still exist and clinical trials are ongoing to answer these questions [6]. For a minority of patients with haemophilia A current ITI strategies are costly, difficult and ultimately ineffective. One study examined the role of race in likelihood of successful immune tolerance and was unable to show a difference but had only a small number of African American (AA) patients [5]. This analysis is particularly important given the higher incidence of inhibitors in the AA haemophilia population [7,8]. Identification of factors which identify patients less likely to achieve tolerance prior to the start of ITI will help identify patients who may benefit from new approaches to ITI. We have reviewed our centre's experience with ITI over the past 17 years and have identified factors which appear to imply a poorer chance of success with ITI.

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Aim

The aim of this study was to study the characteristics (including race), treatment and outcome of patients with haemophilia with inhibitors who have undergone ITI at the Children's Hospital of Michigan over the past 17 years (1992–2009).

Patients and methods

Patients

In compliance with local IRB regulations, patient charts and laboratory databases were reviewed and salient

data were extracted. A total of 31 boys underwent 35 courses of ITI at Children's Hospital of Michigan between 1992 and 2009. Patients included in the study had inhibitor titres of >0.6 Bethesda Units (BU mL^{-1}) on three or more tests. A total of 26 courses were in patients with high responding inhibitor patients (>10 BU mL^{-1} prior to ITI), while nine were in patients with low responding inhibitor titres.

Monitoring

Inhibitor assays, expressed in BU mL^{-1}, were performed using the Bethesda method with the Nijmegen modification (after 1996 when low inhibitor titre was detected by Bethesda assay) [9]. Patients underwent routine laboratory surveillance at 3-month intervals once treated as well as specific testing when inhibitors were suspected or at the time of significant bleeding or planned surgery.

Immune tolerance

Immune tolerance induction consisted of doses of FVIII ranging from 50 units kg^{-1} day^{-1} three to four times weekly for low-responding inhibitors. Patients with high-responding inhibitors were treated with doses of 50 units kg^{-1} day^{-1} up to 250 units kg^{-1} day^{-1}. Clotting factor products were given through a central venous catheter in 30 courses, and by peripheral venipuncture in the other five. Eight patients were given plasma-derived FVIII (PD-FVIII) for ITI, 25 patients were given recombinant FVIII (rFVIII) products and two patients were treated with both. Once ITI was initiated, inhibitor titres and FVIII recovery were measured at 2-week intervals. When inhibitor titres fell to <0.6 BU mL^{-1} on three consecutive measures, the dose of factor product was reduced by half. If subsequent inhibitor assays remained negative (<0.6 BU mL^{-1}), the dose of FVIII was gradually reduced to 25 units kg^{-1} day^{-1} three times weekly.

Successful ITI was defined as inhibitor titre <0.6 BU mL^{-1} on three consecutive tests with plasma FVIII recovery >66% of expected and half-life >6 h. Failure was defined as failure to achieve success in 33 months, or the absence of 20% reduction in inhibitor titre over a 6-month period after the initial 3 months of ITI as defined by DiMichele [10]. Additionally, three patients had to stop ITI because of impaired venous access in the face of rising titre inhibitors 6–10 weeks after initiation of treatment. Low-dose ITI regimen was defined as 50 U kg^{-1} 3–4 times per week while high-dose ITI regimen was defined as 50 U kg^{-1} day^{-1} or higher daily doses.

Statistical methods

Values were calculated using Microsoft Excel 2007 with the @ Risk software (Palisaded corp., Ithica, NY, USA) from palisade decision tool plug-in and consisted of simple descriptive statistics such as mean, median, maximum and minimum as well as univariate *t*-tests, chi-squared analysis and logistic regression analysis.

Results

Race

A total of 31 boys with severe haemophilia A and FVIII inhibitors underwent 35 courses of ITI. Of the 35 courses of ITI in haemophilia A, eight courses were in caucasian patients (22.9%), 23 in AA (65.7%) and four in Middle Eastern patients (11.4%). Of the 31 completed courses of ITI, 22 patients achieved complete tolerance (71%), nine courses failed (29%) and four patients continue on therapy. The length of successful course of ITI was not different between AA and non-AA patients (229.8 vs. 152.4 days, $P = 0.49$, *t*-test) (Table 1). Courses of ITI in AAs, however, were significantly less likely to achieve success than courses in non-AAs (57.9% vs. 92%, $P = 0.04$). This difference was largely attributable to the higher inhibitor titres at the start of ITI in AA patients but race continued to be a predictive factor in a logistic regression analysis including known risk factors for unsuccessful ITI including inhibitor titre at the start of ITI, historical peak inhibitor titre, age at diagnosis of inhibitor and time from diagnosis of inhibitor to start of ITI (Table 2). Using these factors, logistic regression was able to predict success in 100% of our successful patients and failure in 66.7% of our unsuccessful patients for an overall 90% accuracy.

Age, exposure and time to start of ITI

The age at which patients developed an inhibitor ranged from 2 months to 11 years 9 months (median 13 months). Patients who failed ITI had a trend towards earlier development of inhibitor with a mean age at development of inhibitor for those who failed ITI of 1 year 3 months compared to 2 years 4 months in those who were successfully tolerized ($P = 0.055$, *t*-test). The median age at start of ITI was 4 years 8 months (range 7 months to 17 years 11 months).

Table 1. Racial differences in predictive variables for immune tolerance induction.

	African American	Non-African American
Historical peak (BU mL^{-1})	175.7	37.3
Start ITI (BU mL^{-1})	35.2	3.5
Peak on ITI (BU mL^{-1})	196.5	80.3
Time to tolerance (days)	229.8	152.4
Age at the start ITI (months)	65.2	95.9
Age at inhibitor development (months)	15.6	37.1
Exposure days prior to inhibitor	7.8	18.7

ITI, immune tolerance induction.

Table 2. Logistic regression race is a predictive factor for immune tolerance induction outcome.

Summary measures							
Null deviance	36.65185812						
Model deviance	20.033914						
Improvement	16.61794413						
P-value	<0.0001						
Regression coefficients	Coefficient	Standard error	Wald value	*P*-value	Lower limit	Upper limit	Exp(Coef)
Constant	1.668178091	1.694270332	0.984599718	0.3248	−1.652591758	4.988947941	5.302498324
Race	−0.314872272	1.367963791	−0.230175882	0.8180	−2.996081302	2.366336759	0.729882095
Inhibitor at the start of ITI	−0.047770543	0.040671052	−1.174558839	0.2402	−0.127485805	0.031944718	0.953352515
Historical peak inhibitor	−0.006692646	0.008693179	−0.769873286	0.4414	−0.023731277	0.010345984	0.9933297
Time to start of ITI	0.011165283	0.172683948	0.064657331	0.9484	−0.327295255	0.349625821	1.011227848
Age at inhibitor development	0.603191222	0.888816448	0.678645432	0.4974	−1.138889016	2.345271459	1.827942873
Classification matrix	1	0	Percent correct (%)				
Successful	21	0	100.00				
Failure	3	6	66.67				
Summary classification	Percent						
Correct	90.00						
Base	70.00						
Improvement	66.67						

ITI, immune tolerance induction.

The median number of exposure days prior to inhibitor development was 10.5 (1–47). The median time between development of an inhibitor and initiation of ITI was 33 months (range 0–167.1). Delay of more than 1 year between in inhibitor development and initiation of ITI was not associated with poorer likelihood of success (64% <1 year, 74% >1 year, $P = 0.56$ by chi-squared analysis) and time from development of inhibitor to start of ITI did not correlate with lower chance of successful ITI ($P = 0.44$, univariate *t*-test).

Inhibitor titre and regimens

Nine patients had low titre inhibitors (0.6–7.3 BU mL^{-1}) and eight successfully completed ITI using a low-dose ITI regimen of factor infusions 3–4 times per week of 50 U kg^{-1}, one continues on therapy (Table 3). A total of 22 patients with historical peak inhibitor titres of >10 BU mL^{-1} (median 81 BU, range: 10.7–1280 BU mL^{-1}) were treated with daily infusions of 50–250 units kg^{-1} of FVIII products. The mean historical peak inhibitor titres were 46.8 BU mL^{-1} in the tolerized group and 296.3 BU in those who failed ITI ($P = 0.02$, *t*-test). In only seven of the 22 successful trials of ITI did the patient have an inhibitor titre >50 BU mL^{-1} (Table 4). In the unsuccessful trials of ITI, seven of nine had inhibitor titres >50 BU mL^{-1} (Table 5). Higher inhibitor titre at the start of ITI was associated with failure; 11 trials began ITI with inhibitor titres in excess of 10 BU mL^{-1} and seven of them failed while 15 of the 17 trials which began with inhibitor titres below 10 BU mL^{-1} were successful. For ITI, eight trials employed high-purity PD-FVIII/VWF-containing PD products, 25 received rFVIII and two received both. Patients were given the type of product for ITI that they had previously received with the exception of the patients who received both VWF-containing PD and rFVIII. Those courses were switched to a von Willebrand-containing PD product after initial

Table 3. Low responder inhibitor patients who underwent successful immune tolerance induction.

Trial	Historical peak (BU)	Start of ITI (BU)	Peak on ITI (BU)	Time to tolerance (days)	Age start (months)	Time to start (months)	Exposure days prior to inhibitor	Race
15	3.1	0	0	30	33.3	21.7	4	AA
16	4.6	4.4	44	30	211.2	68.7	–	ME
17	0.6	0.6	2	412	6.2	0	14	AA
18	7.1	1	1.8	104	22.4	6.6	–	AA
19	1.7	0.8	0.8	29	11	0.7	10	ME
20	0.9	0.9	0.9	27	55.3	0	47	ME
21	6.5	6.5	6.5	30	–	–	7	W
22	6.3	1.7	5.1	138	12	4.3	18	W
Median	3.9	1	1.9	30	22.4	4.3	12	
Mean	3.9	2	2.7	100	50.2	17.7	16.7	
Range	0.6–7.1	0–6.5	0–6.5	27–412	6.2–211.2	0–68.7	4–47	

ITI, immune tolerance induction; W, White; AA, African American; ME, Arab American.

Table 4. High responder inhibitor patients who underwent successful immune tolerance induction.

Trial	Historical peak (EU)	Start ITI (EU)	Peak on ITI (EU)	Time to tolerance (days)	Age start (months)	Time to start (months)	Exposure days prior to inhibitor	Race
1	54	3.2	95	132	58.2	11.8	14	W
2	11.2	1.5	9.2	55	79.4	24.9	4	AA
3	153	5.9	388	241	57.6	18.3	13	AA
4	34	20	20	50	19.3	13.1	14	W
5	30	11	11	45	12.7	7.3	3	AA
6	28	0	0	69	218.6	95.8	–	ME
7	90	0.7	1.6	88	207	19.3	–	W
8	45	6.9	224	536	138.4	12.2	–	W
9	208	11	139	456	39.9	13.4	8	AA
10	57	1.7	2.3	89	152.3	73	–	AA
11	160	1.8	1.9	60	202.9	12.6	–	W
12	10.7	1.3	2.6	59	79.5	8.4	13	AA
13	24	22	22	49	28.7	15.4	12	AA
14	93	2.8	43	333	141	101	6	AA
Median	44.5	3	15.5	78.5	79.5	14.4	12.5	
Mean	71.3	6.4	68.5	161.6	102.5	30.5	9.7	
Range	10.7–208	0–22	0–388	45–536	12.7–218.6	7.3–101	1–14	

ITI, immune tolerance induction; W, White; AA, African American, ME, Arab American.

Table 5. High responder inhibitor patients who underwent unsuccessful immune tolerance induction.

Trial	Historical peak (EU)	Start ITI (EU)	Peak on ITI (BU)	Duration of trial (days)	Age start (months)	Time to start (months)	Exposure days prior to inhibitor	Race
23	527	59	59	46	15.6	12.3	9	AA
24	16	1.6	1084	730	33.9	21.3	22	W
25	93	28	43	333	66.7	12.4	6	AA
26	18	11	43	66	16.6	13.3	6	AA
27	207	207	207	195	4.4	3.7	6	AA
28	207	7.1	147	713	124.7	120	6	AA
29	84	84	124	107	2	1.6	1	AA
30	1280	66	714	57	139.3	36.5	4	AA
31	235	94	221	180	149	123	3	AA
Median	207	62.5	177	187.5	50.3	17.3	6	
Mean	296.3	62	293.6	269.7	61.4	38.2	7	
Range	16–1280	1.6–207	40–1084	46–730	2–149	1.6–123	1–22	

ITI, immune tolerance induction; W, White; AA, African American.

poor response with rFVIII. Fourteen of 21 patients who received only rFVIII were successfully tolerized compared to seven of eight who received VWF-containing PD products ($P = 0.22$, chi-squared). An analysis of the effect of dosing regimen on chance of success or length of ITI was not valuable in this group as there was a considerable selection bias with low risk patients who were very likely to respond well to ITI being treated with lower doses of factor.

Length of ITI

In patients who became tolerized to FVIII, the average time to achieve an inhibitor titre of <0.6 BU mL^{-1} was 69.5 days (14–1043 days). There was no difference in time to tolerance between AA patients and non-AA patients ($P = 0.24$, *t*-test). In those who did not achieve tolerance, the mean length of the trial was 269.7 days. Twenty-eight of the ITI trials employed a central venous catheter and in five patients ITI was interrupted because of recurrent infections.

Bypassing agents and prophylaxis

In 19 of the trials, the boys received FEIBA, rFVIIa or porcine FVIII for bleeding episodes during ITI; eight of them failed ITI and four are still on therapy. All but two patients who successfully completed ITI went on prophylaxis with FVIII. All patients who successfully completed ITI have maintained tolerance with a mean follow-up of 136 months (range: 6–185).

Anamnestic response

The mean rise in inhibitor titre 2–4 weeks after the start of ITI (anamnestic response) was 37.5 BU mL^{-1} (standard deviation 95 BU mL^{-1}) for those who were successful and 166.1 for those who failed ITI, although

there was considerable variability within groups and this difference was not statistically significant ($P = 0.22$, *t*-test).

Discussion

Risk factors for inhibitor development

It has been well established that the risk of developing inhibitors is highest during the first few exposures to factor products [11–13]. The median number of exposure days prior to development of an inhibitor has been consistently low in several series (9–10 exposure days). This was true in our population with a median number of exposure days prior to inhibitor development of 10.5.

Family history has been associated with inhibitor development independent of the genotype. Identical twins appear to have higher concordance of inhibitor development than do non-twin brothers who in turn have higher concordance than cousins [14,15]. It has been hypothesized that this may be related to polymorphisms in cytokines and differences in MHC complexes which would be more likely shared in more closely related individuals [16]. In our study, five of the patients had a family history of inhibitor development in brothers or cousins.

Early reports have suggested that younger age at first exposure to factor products lead to increased risk of inhibitor development [17,18]. However, these differences may reflect the increased risk of early bleeds in the same group of patients who were at high risk of inhibitor development because of their genotype. Others have proposed that early exposure to factor may induce immune tolerance [19]. A small prospective study examining the use of rFVIIa during the first 2 years of life to avoid FVIII exposure did not seem to result in prevention of inhibitors [20]. A more recent study of larger cohorts has not seen a difference in inhibitor development associated with early age at first exposure [21]. This was also true among patients harbouring the intron 22 inversion mutation. In our patients, the median age at first exposure to FVIII replacement was 6 months (range: 4 days to 51 months).

Patients of African or Hispanic descent have been found to have higher risks of inhibitor development [8,22]. This is true even when comparing patients with identical factor VIII mutations. It has been hypothesized that differential distribution of polymorphisms in cytokines and variations of MHC complexes may account for this increased risk. More recently reports have suggested that polymorphisms in FVIII itself may differ considerably by race with six identified haplotypes. The most commonly used recombinant factor products are of the two haplotypes most common in caucasians. Twenty-four percentage of AAs were found to be of *F8* haplotypes not found in recombinant products, H3, H4 and H5 and patients with these haplotypes had higher inhibitor incidence [23]. Although in patients with mutations causing severe haemophilia very little protein is expressed and what is expressed may not contain the sites of these polymorphisms, it has been hypothesized that clinically undetectable intracellular products of these mutant genes may provide some immunomodulatory effect.

Risk factors for unsuccessful ITI

Our data suggest that in addition to developing inhibitors more frequently, AAs are less likely to respond to ITI. In our cohort, AAs were significantly less likely to achieve tolerance. This remained a predictive factor even when other variables known to effect the likelihood of successful ITI were accounted for a logistic regression analysis. It is possible that genetic variables such as cytokine polymorphisms or FVIII haplotype differences could account for the lower success rates seen in AAs in our study. Larger samples of inhibitor patients will be needed to answer these questions.

Increased time from identification of an inhibitor to the start of ITI was identified as a possible risk factor for failure of ITI [24]. However, this was not corroborated by other large studies [5,25]. We observed no correlation between the time interval between inhibitor development and the start of ITI and successful ITI. The mean interval was 25.8 months in the tolerized group and 38.2 months in those who failed ITI.

Historical peak inhibitor titre has been shown in several studies and a large meta-analysis to correlate with ITI failure [5,24,26,27]. An historical peak titre >200 BU mL^{-1} has generally been employed as the threshold for higher risk of failure; however, the risk of failure appears to increase as titres increase [25,27]. Low-responding inhibitors with peak values <10 BU mL^{-1} have been reported to respond favourably to ITI and often are treated with low-dose ITI protocols with success. In our study group, the historical peak titre was an important predictor of successful ITI. The mean historical peak inhibitor titres were 46.8 BU in the tolerized group and 296.3 BU in those who failed ITI ($P = 0.02$, *t*-test). In only seven of the 22 successful trials of ITI did the patient have an inhibitor titre >50 BU. In the unsuccessful trials of ITI, seven of nine had inhibitor titres >50 BU.

In numerous studies, an inhibitor titre below 10 BU mL^{-1} at the start of ITI has been found to correlate with improved outcomes [5,25,28–30]. Based on these findings, published guidelines often suggest delaying ITI until the inhibitor titre has fallen to <10 BU mL^{-1} [31–34]. In view of the evidence that high titres at the start of ITI increase the risk of ITI failure, the time to start of ITI is often delayed until the titres have fallen below 10 BU mL^{-1}. A recent report shows delaying the start of ITI until the inhibitor titre

drops below 10 BU mL^{-1} results in a median delay of 6 months [6]. During these delays, FEIBA is to be avoided as it contains small amounts of FVIII and may lead to an anamnestic response in inhibitor concentration. In our study, higher inhibitor titre at the start of ITI was associated with failure; 11 trials began ITI with inhibitor titres in excess of 10 BU mL^{-1} and seven of them failed while 15 of the 17 trials which began with inhibitor titres below 10 BU mL^{-1} were successful.

In a retrospective, multicentre, multinational registry, older age at the start of ITI was associated with worse outcomes [24]. We did not see this effect in our study population. The mean age of children successfully tolerized was 83.5 months compared to 61.4 months in those who failed ITI.

Problems with central venous access devices (CVAD) including infection, line failure and thrombosis are commonly result in delays and increase inhibitor titres during ITI. These subsequently decrease the likelihood of successful ITI. It is recommended that if possible ITI be given through a peripheral vein. Given the frequency of access necessary and the young age of the patients, this is often difficult and CVADs are often required.

The dose of factor infused for ITI has varied greatly among the published studies, ranging from 25 units kg^{-1} every other day to 300 units kg^{-1} day^{-1}. Higher doses have been associated with shorter time to achieve tolerance and greater likelihood of achieving tolerance in retrospective studies [24]. There has been considerable debate regarding the optimal dose to achieve tolerance safely and at reasonable cost. A randomized, controlled, multicentre trial to test the efficacy of low- vs. high-dose ITI in a group of patients selected as low risk based on their historical peak titre is ongoing. In our study, low-responding inhibitors were treated with 25 units kg^{-1} every other day with very good success. High-responding inhibitors were treated with 100 units kg^{-1} day^{-1} and doses were increased up to 250 units kg^{-1} day^{-1} in patients who were responding poorly. Because our treatment dose was heavily biased by the patient's likelihood to achieve tolerance, we are unable to evaluate the effect of dose in our study.

von Willebrand factor-containing factor products have recently been reported to improve the chance of successful ITI. However, the data are limited and no randomized trials comparing rFVIII to VWF-containing factors head to head have been completed, although an international study has been initiated. In our group there was a trend towards better results with VWF-containing PD products compared with rFVIII; however, our sample was underpowered to detect a difference.

For symptomatic haemorrhages in patients with high responding inhibitor titres (whether on ITI or not), rFVIIa and activated prothrombin complex concentrates (APCCs) have been the mainstay of therapy. Given that major bleeds during ITI may compromise the chance of successful tolerance, the use of these agents prophylactically to avoid bleeds while on ITI as an adjunctive therapy has been proposed, although to date evidence comparing this strategy to on-demand use of bypassing agents has been published [35].

There is a paucity of literature examining repeated trials of ITI in patients who have failed trials of ITI, although there exists a consensus that the chance of successful tolerance in this group is low. In this study, we have one patient who has failed ITI twice and is currently on therapy with his third trial and has so far had a good partial response with inhibitor titres in the single digits. We have two additional patients who have previously failed ITI once and are on therapy for their second trial. One, whose recent inhibitor titres have been <2 BU mL^{-1} while the other patient's inhibitor titre is above 100 BU.

Conclusions

Most patients with haemophilia A were able to achieve and maintain tolerance (71%). Higher historical peak inhibitor titres, AA race, higher inhibitor titre at the start of ITI and early age at diagnosis of inhibitor were the risk factors for ITI failures. Further study is needed to recognize the patients at the highest risk of failing ITI and to identify the best strategies for improving the chances of successful ITI in them. Particularly in the light of the recent discovery of haplotype differences between ethnicities, further investigation into reasons that AA patients have less success with ITI may not only help this group of patients but may also offer insight into mechanisms that lead to failure of ITI.

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Disclosures

The authors have no conflicts of interest to declare.

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