Immune tolerance induction with recombinant factor VIII in hemophilia A patients with high responding inhibitors

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Immune tolerance induction (ITI) eradicates inhibitors in patients with hemophilia A. This study was designed to investigate the success rate of ITI in high-responding inhibitor patients with severe hemophilia A using recombinant factor VIII (rFVIII). Twenty-six patients received different ITI regimens until a normal recovery (>66%) and half-life (>6 h) of infused FVIII was achieved. In order to maximize the chance of success, the initiation of ITI was deferred in the majority of patients until the inhibitor declined to <10 BU. Twenty-two patients (85%) had baseline inhibitor levels <10 BU (median 2.3 BU) when ITI began. Within a median of 6 months, immune tolerance was achieved in 19 of 26 patients (73%) including 12/17 (70%) with intron 22 inversion, 5/7 (71%) with other null mutations and two with small deletion/insertions in the *F8* gene. In conclusion, recombinant FVIII induces a high rate of immune tolerance even in carriers of null *F8* mutations.

Key words:hemophilia A, FVIII inhibitors, immune tolerance induction.

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In approximately 30% of patients with severe hemophilia A an immune response is mounted towards therapeutically administered factor VIII (FVIII), leading to the production of inhibitor antibodies which neutralize FVIII coagulant activity.[1] Patients with high titer inhibitors require treatment with bypassing agents that help to achieve satisfactory hemostasis but have not been demonstrated to be able to prevent arthropathy and disability. Eradication of the inhibitor by immune tolerance induction (ITI) is generally accepted as the best treatment option because it permits the resumption of FVIII replacement therapy and prophylaxis of bleeding.[2] Several ITI regimens have been used to eradicate FVIII inhibitors and have been shown to be successful in approximately 70% of patients.[3-9] However, the optimal ITI schedule has not yet been agreed upon and the choice of FVIII products to achieve inhibitor eradication is still a matter of debate. Some data suggest that plasma-derived concentrates rich in von Willebrand factor (VWF) increase the success rate of ITI regimens.[10] However, relatively few data exist on ITI using recombinant FVIII (rFVIII).[11-13] On this background, this study was carried out in two large Italian centers with the primary goal to evaluate the success rate of ITI using rFVIII in patients with high responding inhibitors. We also obtained data on the relationship between *F8* gene mutations and ITI outcome and evaluated other factors influencing outcome.

Design and Methods

Starting in April 1996, 26 patients with severe hemophilia A (FVIII:C < 1 IU/dL) and high-responding inhibitors (>5 BU) underwent ITI in two Italian Hemophilia Centers, which consecutively enrolled all the 20 patients who developed an inhibitor after their first exposures to rFVIII. In addition, six patients with long-standing, high-responding inhibitors were treated. No patient had previously undergone ITI. The ITI regimens ranged from 50 FVIII IU/Kg every other day to 200 IU/kg kg-1 daily, with 17 patients (65%) receiving 100 IU/Kg daily. The same product that had induced the inhibitor onset was used in the 20 previously untreated patients who had received rFVIII as their first and only treatment product. No patient received immunomodulatory drug therapy during the ITI treatment. The rFVIII was administered through a peripheral vein access in 14 patients, via an internal arteriovenous fistula (AVF) in five patients and via subcutaneous ports or central venous access devices in seven patients. The use of these last devices was limited by infectious or thrombotic complications in three cases, so that in two of them an AVF was created as an alternative access. The concomitant presence of the following criteria was used to define the ITI as successful: i) no detectable inhibitory activity; ii) normalization of *in vivo* FVIII recovery (> 66%); iii) half-life of infused FVIII > 6 hrs. Partial success was defined by no detectable inhibitor activity without normalization of the *in vivo* recovery (< 66%) and half-life (< 6 hrs) of FVIII. Failure was defined by no decrease of the inhibitor over a 6-month period after the first 3 months of ITI. Both centers used the Bethesda method for the inhibitor test and introduced the Nijmegen modification[14] in 1997. *In vivo* recovery was calculated after a 3-day, treatment-free, wash-out period,

measuring FVIII plasma levels before and 30 min after a challenge dose of 50 IU/Kg of rFVIII. The half-life of FVIII was estimated measuring FVIII levels 15 min, 1, 2, 4, 6, and 24 h after 50 IU/Kg of rFVIII. For patients receiving B-domainless- rFVIII during the ITI treatment the rVIII:SQ standard was used in the one stage assay.

F8 genotyping was carried out using standard methods such as Southern blot and long range polymerase chain reaction (PCR) for inversion analysis, PCR and mutation screening methods, e.g. conformation sensitive gel electrophoresis (CSGE) followed by DNA sequencing, and direct DNA sequencing of the *F8* gene.

Results and Discussion

The main clinical and laboratory characteristics of the 26 patients are summarized in Table 1. The median age at the onset of ITI was 4.2 years (range 0.9-25). Six patients were diagnosed with a FVIII inhibitor more than 2 years prior to the initiation of ITI with intervals of 6.2, 2.2, 5.5, 17.3, 10.7 and 8.7 years between the diagnosis of inhibitor and starting ITI. The remaining 20 patients started ITI as soon as a high-responding inhibitor was diagnosed, although ITI initiation was deliberately deferred until the inhibitor had declined to less than 10 BU in 16 of them (62%) and to less than 5 BU in 13 patients (50%). In these patients the median interval between inhibitor diagnosis and the onset of ITI was 11 months (range 0.8-35). Four patients required an earlier intervention because of frequent or life-threatening bleeds, which prevented delaying the start of ITI. Overall, 22 patients (85 %) had baseline inhibitor levels < 10 BU (median 2.3 BU; range 0-8.5 BU) when ITI began.

ITI was successful in 19 of 26 patients (73%) (Table 2). In these patients the time for the inhibitor to become undetectable ranged from 1 to 38 months (median 3 months), the time to normalization of FVIII half-life from 2 to 40 months (median 6 months), and the time between no detectable FVIII inhibitor activity and normalization of FVIII half-life from 1 to 19 months (median 3 months). ITI was partially successful in two patients. At the onset of ITI, the baseline inhibitor levels of these two patients were 60 and 28 BU. In one of them *in vivo* recovery and half-life of infused FVIII did not normalize, while in the other patient *in vivo* recovery became normal (67%) after 29 months but the half-life of infused FVIII was still shorter than 6 h after 7 additional months of treatment. A feature common to both the partial responders was the occurrence of adverse events during ITI, because their subcutaneous ports were complicated by infection and hematoma that required the interruption of ITI in one of them. In both the partial responders, the inhibitor was no longer detectable after the creation of a proximal AVF in the forearm permitted regular continuation of ITI.

In five patients ITI was a failure and treatment was stopped after 9-21 months, at a time when inhibitor levels ranged between 32 and 1600 BU. There were no adverse events that could apparently have been related to the ITI regimen or the administration of rFVIII.

Table 1. Clinical, laboratory and genotypic characteristics of 26 hemophilia A patients with high-responding inhibitors.

	Patients N.
Plasma FVIII < 1% (%)	24 (92)
Plasma FVIII = 1% (%)	2 (8)
Italian Caucasian origin (%)	25 (96)
Unrelated cases (%)	22 (85)
Related cases (%)	4 (15)
Family history of inhibitors (%)	6 (23)
Age at first FVIII infusion, months* (range)	12 (0.2-63)
Age at inhibitor development, years* (range)	2.2 (0.3-7.7)
Time interval between initiation of FVIII therapy and inhibitor development, months* (range)	9 (12 days- 5 years)
Days of exposure prior to inhibitor development* (range)	14 (6-86)
On demand therapy prior to inhibitor development (%)	24 (92)
Factor VIII product administered prior to inhibitor development	
Plasma-derived FVIII (%)	5 (19)
Recombinant FVIII (%)	21 (81)
Treatment of bleeding episodes after inhibitor diagnosis, prior to ITI	
rFVIIa (%)	15 (58)
aPCC (%)	3 (11)
FVIII (%)	8 (31)
F8 gene mutation	
Int22inv (%)	17 (65)
Int1inv (%)	1 (4)
Large deletion (%)	3 (11.5)
Nonsense (%)	3 (11.5)
Small deletion or insertion (%)	2 (8)

**Median.*

Results of *F8* genotyping indicated that of 17 patients with intron 22 gene inversion 12 successfully to ITI (70%), two responded partially (12%) and ITI failed in three (18%). ITI was also successful in five of seven patients (71%) with other null mutations (one with intron 1 inversion, two with nonsense mutations and two with a large deletion) and in two patients with a small deletion or a small insertion. ITI failed in one patient with a large deletion and in one with a nonsense mutation. The times taken to reach undetectable levels of inhibitor and for FVIII half-life to normalize were similar in patients with intron 22 inversion and in those with other null mutation types (Table 2). All patients who were successfully tolerized or achieved a partial response continued on prophylactic rFVIII replacement therapy. The median time of follow-up after ITI cessation is currently 5.3 years (range 0.1-8.3). In only one patient the inhibitor recurred 7 years after successful ITI. This patient was successfully managed with a second course of ITI.

Since the introduction of ITI in the early 1980s, the success rate achieved with various regimens has varied from 63% to 89% over a wide range of times, from 1 to 28 months.[3-9] It has been surmised that the success of ITI may be positively related to the VWF content of plasma-derived FVIII concentrates. In the experience of

Table 2. Course of inhibitor and outcome of immune tolerance induction.

Patient	*F8 gene mutation*	*Age at ITI start (years)*	*Highest inhibitor level prior to ITI (BU/mL)*	*Baseline inhibitor level at onset of ITI (BU/mL)*	*ITI treatment concentrate*	*Dose of rFVIII (IU/Kg) and frequency*	*Highest anamnestic response at ITI (BU/mL)*	*Months to negative INH test*	*Months to normal half-life*	*ITI outcome*	*Follow-up since ITI success (years)*
1	int22inv	1.9	13	13	Kogenate®	75/day	None	3	5	Success	8.3
2	small deletion	10.1	7	0	Recombinate®	50 QOD^	1	5	6	Success	7.6
3	nonsense	3.8	18	7	Kogenate®	100 QOD^	None	3	4	Success	7.7
4	int22inv	0.9	12	12	Kogenate®	100/day	17	2	5	Success*	7.0*
5	int22inv	7.3	190	1	Recombinate®	100/day	2	2	6	Success	7.4
6	int22inv	4.7	29	4	Recombinate®	100/day	None	2	7	Success	7.2
7	int22inv	2.2	12	0	ReFacto®	200/day	89	N/A	N/A	Failure	N/A
8	int22inv	3.9	13	2	Recombinate®	100/day	None	1	2	Success	7.2
9	large deletion	4.3	60	4	Kogenate®	100/day	16380	N/A	N/A	Failure	N/A
10	int22inv	25.0	512	8	Kogenate®	100/day	4096	N/A	N/A	Failure	N/A
11	small insertion	14.6	117	4	Recombinate®	100/day	8	1	2	Success	6.8
12	large deletion	4.2	10	8	Kogenate®	100/day	None	3	5	Success	6.5
13	int22inv	0.9	60	60	Recombinate®	100/day	70	15	N/A	Partial Success	5.3
14	int22inv	1.0	8	4	Recombinate®	200/day-133m 100/day-1 7m	8	37	39	Success	3.2
15	int22inv	10.8	86	0	Recombinate®	100/day	3	1	4	Success	5.9
16	large deletion	3.8	105	4	Recombinate®	100/day	10	4	6	Success	5.3
17	int22inv	4.5	100	2	Kogenate®	100/day	105	5	10	Success	5.0
18	int22inv	5.6	500	3	Kogenate®	100/day	4	4	8	Success	5.0
19	int22inv	6.7	10	2	Recombinate®	100/day	None	1	20	Success	3.0
20	nonsense	4.2	15	1	ReFacto®	60 QOD	7	6	13	Success	1.8
21	int22inv	4.6	64	0	Kogenate®	100/day	128	3	6	Success	2.4
22	int1inv	2.3	175	1	Recombinate®	60 QOD 9m 100 QOD 6m 100/day 8 m	600	21	23	Success	0.9
23	int22inv	1.2	45	28	Kogenate®	100/day	800	29	N/A	Partial Success	0.1
24	nonsense	8.8	128	7	Recombinate®	100/day 12m 200/day 7m	512	N/A	N/A	Failure	N/A
25	int22inv	2.5	70	6	ReFacto®	200/day	950	N/A	N/A	Failure	N/A
26	int22inv	3.7	128	1	Recombinate®	200/day	1770	10	18	Success	0.2

^*Every other day; *patient relapsed 7 years after cessation of ITI while he was continuing to receive prophylactic treatment with rFVIII; N/A: not applicable.*

a German center, the success rate was indeed significantly higher when concentrates containing large amounts of VWF (88%) were used rather than monoclonal or rFVIII (28%). In addition 80% of patients unresponsive to ITI carried out with monoclonal or rFVIII achieved tolerance when they were switched to plasma-derived VWF/FVIII concentrates.[10] Our study shows that ITI can be successfully achieved with rFVIII. The success rate and duration of treatment were comparable to those reported by international registries,[15-18] although it must be recognized that potential outcome variables may be different in different studies. Definite conclusions about the best type of concentrate for ITI courses should be obtained through randomized, multicenter trials that are difficult to conduct for a variety of reasons: inhibitors develop mostly in children, who are generally treated with rFVIII products; many patients refuse plasma-derived products and concentrates containing large amounts of VWF are becoming less available in many countries.

From the largest studies reported so far, it is apparent that a number of variables may contribute to the likelihood of success, the most consistent of which is a low inhibitor level at the onset of ITI.[7,15-18] In this study the start of ITI was deferred until the inhibitor level had declined to less than 10 BU by avoiding boosting the inhibitor with FVIII-containing products during the interval between diagnosis and the start of ITI. Results indicate that initiation of ITI can be postponed without mishaps in young patients, especially when poor venous access may cause a temporary interruption of regular ITI. Interruption of ITI and intercurrent infections have been considered causes of unsuccessful ITI.[9,17] Since catheter-related infections occurred in the two patients who responded only partially to ITI, our results support the view that intercurrent infections, which cause non-specific stimulation of the immune system, may be detrimental during ITI.[9] These findings also corroborate the suggestion of using internal AVF in children with poor peripheral vascular accesses who need to start a course of ITI.[19] On the whole, it appears that the start of ITI can be delayed in young patients until the inhibitor level decreases to less than 10 BU and a good venous access is found.

The results of this study, in which all the patients were genotyped, provide some information on the influence of *F8* gene mutation on ITI outcome, a yet poorly explored issue.[8-9,13] As expected, the genetic status affected inhibitor development, because 92% of patients requiring inhibitor eradication had null muta-

tions of the *F8* gene and 65% had the intron 22 inversion as a causative mutation. Null mutations, however, did not obviously affect the chance of achieving successful ITI, because 12/17 (70%) of patients with intron 22 inversion and 5/7 (75%) of those with other null mutations were successfully tolerized. Neither was there a tendency towards longer duration of ITI being longer in patients with intron 22 inversion than in patients with other types of mutation, at variance with the observation of Oldenburg *et al.*[9] On the other hand, it must be recognized that the number of patients studied was small, so that these results, which differ from previously, reported findings, should be confirmed in a larger number of patients.

AR, ES: conceived the study. The study was performed in two Italian Hemophilia Centers under the direction of PMM, who also revised the manuscript. AR, MEM and ES collected and analyzed the clinical and laboratory data; AR and ES wrote the manuscript with contributions from other authors. The authors reported no potential conflicts of interest.

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