

Case Report: Induction of Immune Tolerance to Factor VIII Inhibitor after a Major Operation

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Abstract

Introduction: We report a successful case of immune tolerance to factor VIII (FVIII) inhibitor after a major operation. An attempt was made to induce immune tolerance with inhibitor in a haemophilia A patient, who was required to undergo an above-knee amputation. We opted to give high-dose FVIII infusion with no immunosuppression. **Outcome:** The highest preoperative FVIII inhibitor level was 5 BU and the peak postoperative FVIII inhibitor level was 1.5 BU demonstrated on Day 9 post operation. High-dose FVIII support was provided during the perioperative period and continued with a low maintenance dose to achieve a FVIII level of 30% to 40%. The requirement of high-dose FVIII lasted from day 6 to 23 post operation and this was tailed down to a maintenance dose over the next 37 days. There were only 2 episodes of mild oozing from the wound at around Day 9, which coincided with the peak postoperative FVIII inhibitor level. Both bleeding episodes were arrested adequately by administering a single dose of FEIBA during each episode. Immune tolerance was demonstrated after around 3 months and a follow-up period of 233 days showed no recurrence of FVIII inhibitor with the normalisation of FVIII half-life study. **Conclusion:** After immune tolerance, the patient suffered fewer episodes of joint haemorrhage and required a lower amount of FVIII infusion as well. The cost may be high initially but the long-term cost-effectiveness has to be carefully evaluated.

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Case Report

Mr ZBH was a 30-year-old man with a history of severe haemophilia A with 0% factor VIII (FVIII). He was initially supported with cryoprecipitate but was switched to FVIII concentrate in 1995. He first developed FVIII inhibitor in 1995 and his inhibitor level fluctuated between 0.3 and 2 BU. In view of his low inhibitor level, support with FVIII concentrate was continued. However, in 2003, Mr ZBH began to develop a higher inhibitor level, which peaked at 5 BU, and the factor concentrate support was subsequently changed to FEIBA, factor VIIa (FVIIa) or factor IX prothrombin complex. In the same year, he sustained a knee joint infection after the revision of his left knee replacement operation. After much deliberation, above-knee amputation was planned and the challenge was to support him during this operation.

Methods

FVIII Assay

A mixture of FVIII deficient plasma and the patient's

plasma was tested in the partial thromboplastin assay and the result was interpreted using a reference curve obtained with dilutions of standard human plasma or a normal plasma pool mixed with the deficient plasma.

FVIII Inhibitor Assay

Different dilutions of the patient's plasma was mixed with normal plasma and incubated for 2 hours at 37°C. A factor assay was performed on the mixture and this was compared to an assay on normal plasma mixed and incubated with a buffer. 1 BU of inhibitor is defined as the amount of inhibitor required to reduce the normal plasma factor level by 50%.

FVIII Recovery

After a washout period of 72 hours, a single dose of FVIII was given and a blood sample was sent for FVIII level and inhibitor assay 1 hour after infusion. The FVIII recovery is calculated as follows:

% FVIII recovery = Measured FVIII level/Infused FVIII level x 100,

where Infused FVIII level = Unit of FVIII infused x 2/weight.

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FVIII Half-life Study

Similarly, after a washout period of 72 hours, a single dose of FVIII (30 U/kg) was given and repeated FVIII level assays were done at 0 hour, 1 hour, 4 hours, 6 hours, 8 hours and 25 hours. The half-life was calculated as the amount of time for FVIII to fall to half the maximum level recovered. The formulae used are:

$$K_{elim} = [\ln(C_{peak}) - \ln(C_{trough})] / T_{interval}, \text{ and}$$

$$T_{1/2} = 0.693 / K_{elim}$$

Detection of Anti-idiotypic Antibodies

We are very grateful to Dr Jean-Marie Saint-Remy (from the Center for Molecular and Vascular Biology, University of Leuven, Belgium) for kindly agreeing to perform the necessary tests. The specimen was spun, and the plasma separated and shipped to her laboratory for detection of possible anti-idiotypic antibodies.

Definition of Successful Immune Tolerance

Success was defined as the presence of all the following:

- i. No FVIII inhibitor demonstrated.
- ii. Normalisation of FVIII recovery ($\geq 66\%$ at 1 hour post-infusion)
- iii. Normalisation of FVIII half-life study (half-life ≥ 6 hours).

Results

FVIII and FVIII Inhibitor Level (Fig. 1)

Prior to the operation, we conducted a pharmacokinetic study of the FVIII inhibitor, in order to provide ourselves with a useful guide to the potential inhibitor kinetics during the actual operation. A single dose of 1250 units of FVIII was administered and the FVIII inhibitor level was monitored regularly thereafter. After the test dose was administered on Day -72, FVIII inhibitor began to rise immediately from 1.5 BU to a peak of 5 BU approximately 14 days later. It began to decrease gradually to 2 BU over the next 3 weeks and this was followed by a slower phase of decline to 1.4 BU over the next 5 weeks. During this period, all bleeding episodes were controlled with either FEIBA or factor 7a.

Preoperatively, FVIII inhibitor was demonstrated to be 1.3 BU from Day -2, so we started to load the patient with 150% FVIII per 24 hours. The loading dose was started from Day -2 in order to optimise the FVIII level for operation. From Day -2 to Day 0, FVIII level was increased from 0% to 85%. This was deemed to be sufficient for surgery and the operation proceeded as planned without any complications. Postoperatively, FVIII was increased to 200% per 24 hours from Day +2 because FVIII level could be maintained only for the first 2 days and started to decline

from Day +2. However, there was no active bleeding, no FVIII inhibitor was detected and the FVIII level could be maintained above 50%. Therefore, the FVIII infusion was maintained at 200% per 24 hours.

There was a sudden decline in the FVIII level from Day +6, with a resurgence of FVIII inhibitor demonstrated on Day +7. This was expected from the kinetics study conducted preoperatively as the booster effect resulting from the FVIII infusion.

Together with the resurgence of FVIII inhibitor, mild bleeding also started from the stump wound. We decided to increase the dose, similar to the reported Bonn protocol of 400% per 24 hours.¹⁻³ We intended to continue the Bonn protocol until immune tolerance could be achieved, in order for the patient to revert to the use of FVIII in the future.

The FVIII inhibitor did not persist and started to decline over the next 3 days to 0 BU. From Day +12 onwards, there was no longer any FVIII inhibitor detectable until Day +233.

Over the next 10 days from Day +12 to Day +21, FVIII inhibitor remained at 0 BU and the FVIII level began to increase gradually to 66% with a stable dose of 400% per 24 hours.

In view of the good haemostasis and absence of FVIII inhibitor, FVIII dosage could be reduced abruptly over the next 7 days to about 140% per 24 hours, in order to maintain the FVIII level around 30% with 0BU of FVIII. Moreover, FVIII recovery had not been restored to the normalised target level of $>66\%$.

From Day +28 onwards, we could gradually switch to a less intensive regime of 3 doses per week, while maintaining a FVIII level of around 30% with 0BU of FVIII. Thereafter, regular FVIII and FVIII inhibitor were monitored while waiting for the normalisation of FVIII recovery. FVIII recovery began to normalise from around Day +68 and continued to normalise on follow-up.

By Day +110, FVIII recovery was repeatedly normalised and we decided to stop the immune tolerance prophylaxis and to conduct the FVIII half-life study, which demonstrated a normalised half-life of ≥ 6 hours as well. The FVIII recovery was still normalised without any factor inhibitor detected on follow-up to Day +233.

FVIII Half-Life Study

As demonstrated in Figure 2, there is a faster phase which corresponds to the redistribution of FVIII, and a second slower phase corresponding to the elimination of FVIII. Due to the fast redistribution phase, half-life was often determined to be shorter. Therefore, 2 $T_{1/2}$ were calculated: the first $T_{1/2}$ was determined with the peak FVIII level,

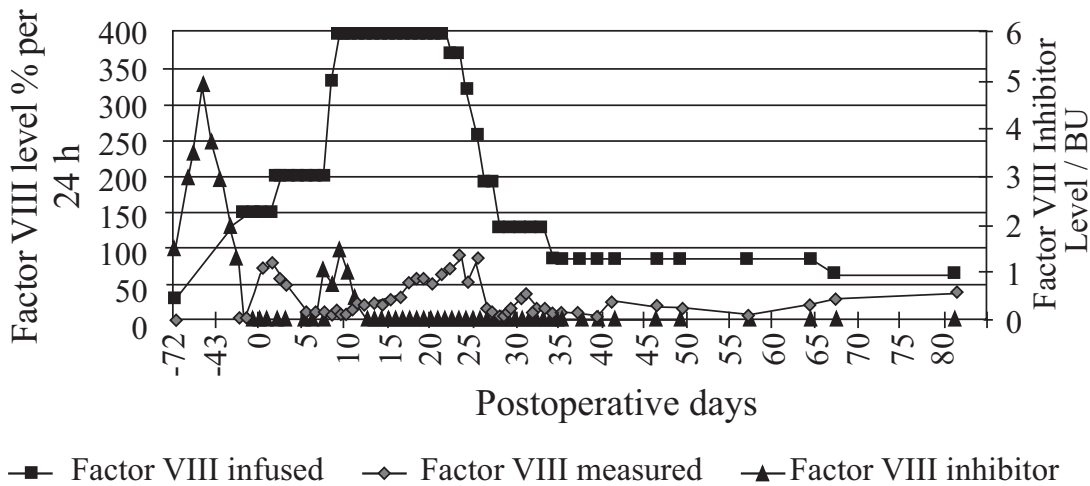


Fig. 1. Level of factor VIII and factor VIII inhibitor from day -72 to +84

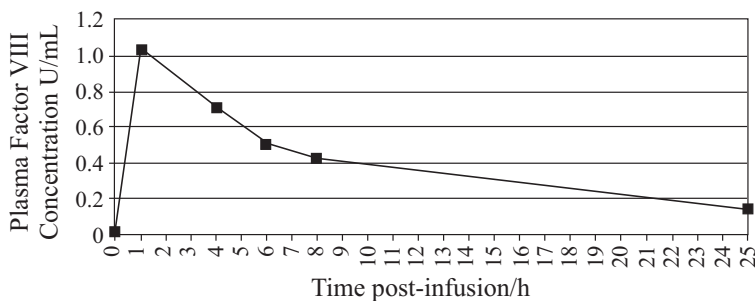


Fig. 2. Half-life study of factor VIII (Day +110).

	Time post-infusion/h	FVIII U/mL	Kelim	T1/2
Total	1	1.03	0.086	8.1
	25	0.13		
Elimination	8	0.43	0.069	10.0
	25	0.13		

while the second T1/2 was determined during the elimination phase. Though both T1/2 were normalised to >6 hours, only the elimination T1/2 was normalised within the normal range in the product information (10 to 14.8 hours).

Haemorrhage Episodes

Mr ZBH had a rather uneventful postoperative recovery except for a slight oozing from the wound on Day +9 and Day +10. He was treated with 2 doses of FEIBA and the bleeding responded well.

One interesting observation was the reduced FVIII requirements after immune tolerance. Before immune tolerance, Mr ZBH generally suffered 1 episode of haemorrhage per week. However, after the tolerance, the number of joint haemorrhages was reduced to an average of 1 episode per month. We could not explain this reduction but it has been documented in other studies as well.

Anti-Idiotypic Antibody Assay

According to tests done in Dr Jean's laboratory, there was no detectable anti-idiotypic antibody demonstrated in Mr ZBH's specimen.

Discussion

The management of major surgeries in Haemophilia A patients with inhibitor is difficult, because there are few

proven treatment options.^{1,2} Options with proven efficacy include FVIII, aPCCs (activated prothrombin complex concentrates) and FVIIa. In view of the association of high-dose aPCCs with myocardial infarction and disseminated intravascular coagulation, only FVIII and FVIIa were considered in this case. Though both FVIII and VIIa have documented efficacy, the use of FVIII has a further advantage of possible immune tolerance induction, especially in low responders. Besides, the estimated cost for a possible high-dose FVIII support was either lower or comparable to that of F VIIa. Therefore, the final decision was to support the operation with FVIII.

In order to induce immune tolerance of FVIII inhibitor, the most common protocols³⁻⁵ include the Bonn protocol of high-dose FVIII, the van Creveld protocol of low-dose FVIII, and the Malmo protocol of high-dose FVIII with immunosuppression. Since Mr ZBH would be undergoing a major operation, we decided to exclude the Malmo protocol, which requires immuno-suppression. However, there are no definitive data on preferential support for either a high-dose or low-dose protocol. Therefore, the decision was made to infuse enough FVIII to maintain a FVIII level of about 100% for the first week, followed by a maintenance dose to induce immune tolerance. In fact, the highest requirement of FVIII was about 400% per 24 hours and it occurred from Day +6 to Day +12 when the

maximum FVIII inhibitor was demonstrated. The dose of 400% per 24 hours is, incidentally, similar to the high-dose FVIII infusion of 100 U/kg bd during Phase 1 of the Bonn protocol. Thus, it may not be necessary to decide on the actual immune protocol to use; instead, the decision could be guided by the requirement of FVIII both to obliterate FVIII inhibitor and to maintain an adequate FVIII level until tolerance is established. Fortunately, Mr ZBH was only a low responder, with a maximum FVIII inhibitor of 5 BU, otherwise the amount of FVIII required might have been too high to be feasible for infusion.

Since FVIII inhibitor increases from Day +6, peaks around Day +9 and decreases to baseline around Day +12, this period of maximum FVIII inhibitor stimulation will probably constitute the highest risk of bleeding. However, the preoperative kinetics study demonstrated a longer period of 5 weeks before the FVIII inhibitor is reduced to the minimum, so the expected period of high bleeding risk may need to be extended to about 5 weeks after initial stimulation. During this period of high haemorrhagic risk, any episode of bleeding can be haemostatically secured with either FEIBA or FVIIa.^{6,7}

Interestingly, Di Michele⁸ has reviewed the results of various immune protocols and shown that the median time to successful tolerance using the Bonn high-dose protocol ranged from 15 to 32 months. For Mr ZBH, the time to successful immune tolerance was 3 months; the actual duration that Mr ZBH was treated with such high-dose FVIII (i.e., 100 U/kg bd) only lasted from Day +7 to Day +21, and was subsequently maintained with about 50 U/kg till tolerance. Thus, both the time to successful tolerance and the amount of factor used was far less than those in the Bonn's protocol. However, there were no corresponding data on possible reduction in duration and FVIII usage in the Bonn protocol for a patient undergoing a major operation. We are interested to know whether the anti-inflammatory milieu during the postoperative repair phase could have enhanced the success of immune tolerance. If the postoperative repair phase does favour a successful immune tolerance, it is possible that other major operations in patients with inhibitor could be regarded as a golden opportunity for the successful induction of immune tolerance. We have now followed this patient for 2 years and there was a consistent absence of FVIII inhibitor with normalised FVIII recovery. The immune tolerance seems to have persisted so far and the patient has been able to revert to using FVIII for all bleeding episodes.

Several studies⁹⁻¹¹ have demonstrated anti-idiotypic antibodies that were associated with successful immune tolerance. Due to the complexity of the test methodologies, we could only send the plasma specimen to Dr Jean-Marie

Saint-Remy's laboratory for identification of such antibodies. The results were negative for such anti-idiotypic antibodies, and therefore the actual mechanism for this successful tolerance has yet to be identified.

The total FVIII used amounted to about 300,000 units and the total cost was about S\$140,000. This is definitely not a meager sum and therefore this approach may not be advocated for all patients. However, if the calculation of cost includes the reduction in the amount of FVIII required after tolerance as well as the reduced episodes of bleeding, it may ultimately prove to be a feasible option.

In conclusion, the key factors that have contributed to the success in Mr ZBH probably include the low responder status, high-dose factor immune tolerance programme and uncomplicated postoperative recovery.

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