

Induction of immune tolerance and suppression of anaphylaxis in a child with haemophilia B by simple plasmapheresis and antigen exposure

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Summary. Anaphylaxis to factor IX (FIX) in patients with haemophilia B is a rare and life-threatening complication that has been reported to occur in association with the development of inhibitors to FIX. Management of these patients is difficult. This report presents an 18-month-old boy with a frame-shift mutation of the FIX gene and FIX coagulant level of <1% who developed anaphylactoid reactions to low and high purity plasma-derived FIX concen-

tration infusions and an inhibitor measuring 1.0 BU mL⁻¹. The patient was managed with simple plasmapheresis, a short course of corticosteroids and high-dose antigen exposure, which successfully induced long-lasting immune tolerance to FIX concentrates.

Keywords: anaphylaxis, antigen exposure, factor IX inhibitor, immune tolerance, plasmapheresis.

Haemophilia B (Christmas disease) is a bleeding disorder resulting from mutations in a single copy of a 34-kilobase gene on the X chromosome (q27.1) coding for a protein known as factor IX (FIX) [1,2] and is clinically indistinguishable from haemophilia A. The severity of clinical manifestations is directly related to the degree of factor deficiency. Patients with severe deficiency experience frequent spontaneous bleeding episodes most commonly in the large joints.

Replacement therapy with plasma-derived, viral-attenuated FIX concentrate and recently recombinant FIX, has dramatically improved the quality of life of these patients. With modern treatment regimens incorporating prophylactic factor replacement, patients can look forward to near normal levels of activity.

In 1–3% of haemophilia B patients [3,4] inhibitors to the replacement factor occurs, resulting in a marked decrease in the functional activity of the replacement therapy. Inhibitors are usually detected when the patient experiences a decrease in the effectiveness of

prophylaxis or treatment of bleeding episodes. Most inhibitors occur in early childhood. Remarkably, some patients with FIX inhibitors experience allergic or anaphylactic reactions to FIX infusion, which greatly complicates their management [5,6].

The preferred treatment of young patients with inhibitors is to induce immune tolerance with frequent and high-dose factor replacement and then to proceed to prophylaxis. This is clearly difficult when treatment is complicated by anaphylaxis. While alternative therapies with activated factor concentrates and recombinant VIIa can be used, they do not provide the opportunity for a prophylactic regimen.

In this paper, we describe the use of a simple and relatively inexpensive plasmapheresis technique to achieve immune tolerance in a patient with haemophilia B who also had anaphylactoid reactions to FIX concentrate replacement therapy.

Patient and methods

An 18-month-old boy was diagnosed with haemophilia B at the age of 8 months after presenting with multiple spontaneous ecchymoses and a spontaneous haemarthrosis of the knee. Coagulation factor assays

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confirmed the diagnosis of severe haemophilia B with a FIX level of <1%. Subsequent investigations of his gene defect revealed a deletion of a C in the pre domain of the leader peptide (83 del C) (Fig. 1). This mutation results in a premature termination at codon -27 and has not been previously reported on the FIX database [7]; however, another has been reported that results in termination at the same codon (89 TA) (see www.ums.ac.uk/molgen/fix1.htm). Figure 1 shows the patient's FIX sequencing at the point of mutation compared with a normal control.

He was treated with 500 units (60 units kg⁻¹) Prothrombinex-HT as a twice-weekly prophylactic regimen administered in the hospital (heat-treated plasma-derived low purity factor II, IX and X; CSL Bioplasma Ltd, Broadmeadows, Victoria Australia).

With his 11th routine treatment with Prothrombinex-HT the patient experienced an episode of flushing, audible wheezing, hypotension and tachycardia. The treatment was ceased and the patient improved with an antihistamine and corticosteroids. A further challenge with Prothrombinex, in a supervised environment, resulted in identical symptoms and an anaphylactoid reaction to Prothrombinex was diagnosed.

Replacement therapy was then changed to highly purified FIX (Immunine, Baxter, Old Toongabbie, NSW, Australia); however, the patient continued to experience flushing, tachycardia and shortness of breath and he required corticosteroid and antihistamine cover during infusions. At the same time, frequent soft tissue bruising and spontaneous joint bleeds were noticed and an inhibitor to FIX measuring 0.55 Bethesda Units (BU) mL⁻¹ was identified. The inhibitor level increased to 1.0 (BU) mL⁻¹ and FIX recovery study showed zero recovery 2 h after a

dose of 80 units kg⁻¹ of Immunine. Immunoglobulins and cellular immune response were normal.

The patient was admitted to the Intensive Care Unit at the Royal Children's Hospital, Melbourne. Informed consent was obtained from his parents for a plasmapheresis procedure and, under cover of intravenous steroids with adrenaline and antihistamine stand-by the patient was challenged with high-dose Prothrombinex. We attempted to ameliorate his anaphylactic response and, by massive antigen exposure, commence immune tolerance induction to his FIX inhibitor.

The patient was sedated with midazolam and morphine infusion. Immunine was used to allow insertion of a large bore intravascular catheter. A standard plasmapheresis filter (Gambro PF 1000) was incorporated into the plasmapheresis circuit.

A total of 2 blood volumes were circulated (approximately 200 mL kg⁻¹, 4% normal serum albumin and 3 units of fresh frozen plasma were used as a replacement fluid as is the standard of care for the plasmapheresis procedure.

Immediately after completion of the plasmapheresis the patient was premedicated with methylprednisolone at 2 mg kg⁻¹, and Prothrombinex at 130 units kg⁻¹ was then infused in the intensive care unit. The patient received daily doses of Prothrombinex, with corticosteroid cover, for the next 4 days in the ward, after which time the intravascular catheter was removed and the patient discharged from hospital. He received further daily infusions (130 units kg⁻¹) with progressively reducing oral steroid cover for 40 days. The regimen was changed to second daily infusions (130 units kg⁻¹) for 2 months and then twice weekly infusions. Despite the presence of an inhibitor the plasmapheresis procedure was uncomplicated and the patient experienced no bleeding with insertion of a large bore plasmapheresis catheter.

Under steroid cover there were no signs of anaphylaxis either during or immediately following after the infusion of Prothrombinex.

During further Prothrombinex infusions the patient experienced no episodes of spontaneous joint bleeds. With a 3-year follow up on a prophylactic regimen, there is no clinical or laboratory evidence of the recurrence of the previous inhibitor. The patient's treatment was changed 2 years after the immune tolerance regimen to a new more highly purified FIX concentrate (Monofix, CSL) without complications.

FIX recovery post-immune tolerance was assayed following infusions of Prothrombinex and Monofix (Fig. 2). FIX half-life calculated for Prothrombinex and Monofix Win-NonLin Standard V2.1 (1998) (Pharsight software; Pharsight Corp., Mountain

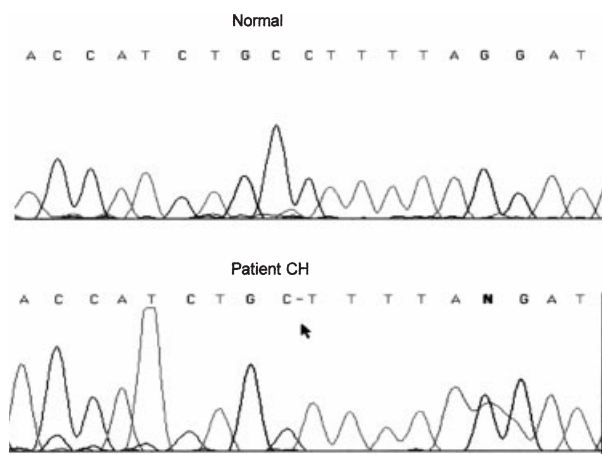


Fig. 1. The patient's factor IX gene sequencing at the point of mutation.

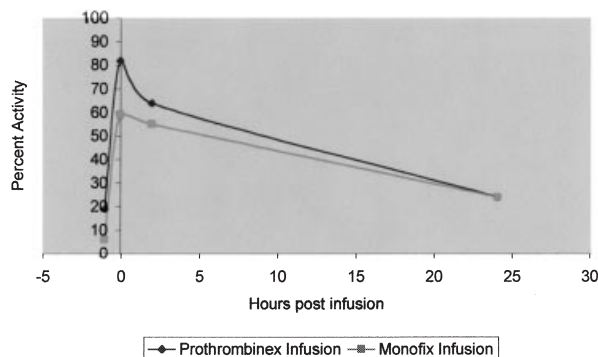


Fig. 2. Factor IX activity following infusion (Prothrombinex and Monofix) post-immune tolerance. Factor IX half-life post-immune tolerance: Prothrombinex infusion terminal half-life 14.0 h (Rsquared adjusted 0.973); Monofix infusion terminal half-life 18.3 h (Rsquared adjusted 1.0).

View, CA, USA) (Fig. 2). The results show a FIX half-life of 14 h following Prothrombinex infusion and a FIX half-life of 18.4 h following Monofix infusion. The increased FIX half-life for Monofix was calculated 12 months after the Prothrombinex half-life calculation and suggests a maintained and improving FIX half-life post-immune tolerance.

Discussion

The association of anaphylaxis and inhibitor development in patients with haemophilia B was first documented by Warrier *et al.* [4] with clinical and laboratory data from 12 haemophilia centres in North America and in Europe. This association appears to be peculiar to FIX deficiency. At present there is only speculation for this relationship, with the most likely reason being the lower frequency of complete absence of FIX synthesis in haemophilia B when compared with haemophilia A. In this context it may be relevant that the mutation in this patient is a frame-shift in the pre domain of the leader peptide resulting in a termination at codon -27. A truncating mutation in the leader peptide would almost certainly result in the non-release of any functional FIX.

The development of an inhibitor and anaphylaxis is a serious complication as it potentially deprives the patient of the benefit of prophylaxis and may necessitate management with more expensive products such as recombinant factor VIIa rather than the cheaper plasma-derived FIX concentrates.

Anaphylaxis in our patient occurred with the 11th dose of Prothrombinex and the inhibitor was noted

subsequently. The Prothrombinex concentrate is of low purity and it is possible that the anaphylaxis was due to other proteins contaminating the concentrate. However, the time association of both anaphylaxis and inhibitor suggest that the immune disturbance was most likely due to the same antigen and the need to use corticosteroids and antihistamines with Immune infusions is further evidence that the immune response was directed at FIX epitopes.

The decision to induce immune tolerance by plasmapheresis, corticosteroids and antigen loading only was based on the work of Nilsson *et al.* [7] with the Malmo protocol for immune tolerance induction. We did not wish to use cyclophosphamide because of its potential long-term toxicity and we did not have access to immunoadsorption columns. The success in eliminating both anaphylaxis and inhibitor suggest that this simplified and inexpensive procedure may be successful in at least some patients soon after diagnosis of this complication and when the inhibitor level as measured by the Bethesda assay is low.

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