

The use of rituximab as an adjuvant for immune tolerance therapy in a hemophilia B boy with inhibitor and anaphylaxis to factor IX concentrate

Ampaiwan Chuansumrit^a, Yingwan Moonsup^a, Nongnuch Sirachainan^a, Suwat Benjaponpitak^a, Atchara Suebsangad^b and Pakawan Wongwerawattanakoon^b

We describe a 10-year-old severe hemophilia B boy with a stop codon mutation of exon 2 in the factor IX gene who developed high inhibitor of 70 Bethesda units (BU) from 12 months of age after exposure to prothrombin complex concentrate for 14 days. The inhibitor spontaneously disappeared within 3 months. The patient, however, exhibited anaphylactic reaction to the administration of prothrombin complex concentrate and factor IX concentrate at ages 15 and 23 months, respectively. Although recombinant activated factor VII was alternatively given, he suffered from progressive hemophilic arthropathy. At the age of 10 years, the boy underwent desensitization to factor IX concentrate and could tolerate factor IX concentrate of 40 U/kg administered on day 9 of desensitization. Unfortunately, the inhibitor of 16 BU was detected on day 6 and rapidly increased to 180 BU on day 9 of desensitization. Rituximab 375 mg/m² per week was therefore immediately initiated on day 10 and a total of four doses were given. The inhibitor gradually decreased to 21.5 BU after the fourth dose of rituximab. The daily factor IX concentrate administration of 40 U/kg was continued for 1 month and

decreased to three times per week for another month, and then to once to twice per week for the remaining 14 months of desensitization. The patient was able to attend regular school and the most recent inhibitor ranged from 4.4 to 10 BU. No proteinuria or alteration of renal function was found. In conclusion, rituximab is a helpful adjuvant to immune tolerance therapy in a hemophilia B boy with inhibitor and anaphylaxis to factor IX concentrate. *Blood Coagul Fibrinolysis* 19:208–211 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Blood Coagulation and Fibrinolysis 2008, 19:208–211

Keywords: anaphylaxis, hemophilia B, immune tolerance therapy, inhibitor

^aDepartment of Pediatrics and ^bDepartment of Nursing, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Correspondence to Prof. Ampaiwan Chuansumrit, MD, Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Rama VI Road, Bangkok 10400, Thailand
Tel: +662 2011748; fax: +662 2011748; e-mail: raajs@mahidol.ac.th

Received 3 July 2007 Revised 7 September 2007
Accepted 26 September 2007

Introduction

Among patients with hemophilia, 85–90% have hemophilia A and 10–15% have hemophilia B. The risk of developing inhibitor differs; 25–30% of hemophilia A patients develop inhibitor to factor VIII clotting activity [1,2], while only 1–3% of hemophilia B patients develop inhibitor to factor IX clotting activity [3]. The latter usually possess large deletions or major abnormalities of the factor IX gene [4,5]. Moreover, some patients experience severe allergic or anaphylactic reaction to the factor IX infusion simultaneously with the appearance of the inhibitor, before or after the occurrence of the inhibitor. The inhibitor complicates the effectiveness of bleeding control, leading to chronic hemarthrosis, deformities or even death. The immune tolerance therapy (ITT) for eliminating the inhibitor is essential for improving the quality of life. Severe allergic or anaphylactic reaction, however, is a barrier for ITT.

Rituximab, a monoclonal antibody against CD20-positive B cells, has shown an effectiveness in hemophiliacs with inhibitor [6–8]. In the present study, we report the effectiveness of rituximab as an adjuvant for ITT in a

boy with hemophilia B inhibitor and anaphylaxis to factor IX concentrate.

Case report

A 10-year-old boy with severe hemophilia B (factor IX clotting activity <1%) and a point mutation at exon 2 (nucleotide 6460, cytosine to thymine) inducing the stop codon was reported. After exposure to prothrombin complex concentrate (Profilnine) for 14 days when aged 9–12 months, the boy developed a high inhibitor titer of 70 Bethesda units (BU) at 12 months of age. After that, recombinant activated factor VII was given to control bleeding episodes. The inhibitor spontaneously disappeared within 3 months. At the age of 15 months, however, the patient developed urticaria, tightness of breathing and cold clammy skin immediately after receiving prothrombin complex concentrate. Adrenalin and hydrocortisone were administered with responsiveness. The administration of prothrombin complex concentrate was thus discontinued and recombinant activated factor VII was given to control the subsequent bleeding episodes. No recurrent inhibitor was found. At the age of 23 months, factor IX concentrate (Alphanine) was tried. The boy also exhibited urticaria and angioedema 15 min

after receiving factor IX concentrate. Adrenalin and hydrocortisone were administered with responsiveness. From the ages of 2 to 10 years, even though the nurturing parents provided the appropriate care for avoiding trauma, the patient experienced a median of eight bleeding episodes per year (interquartile range, 3–12). Due to the high cost of recombinant activated factor VII, the boy received inadequate treatment resulting in progressive hemophilic arthropathy.

After discussion among physicians, parents and the patient, the desensitization to factor IX concentrate (Alphanine) was considered. In cases of the occurrence of inhibitor, the use of rituximab as an adjuvant to ITT was planned. The trial was approved by the Faculty Ethics Committee and informed consent was obtained from the patient and parents.

The patient was admitted to the pediatric intensive care unit. Initially, a skin prick test with factor IX concentrate was performed and revealed a negative result. Histamine was used as a positive control and normal saline as a negative control. Successively, intradermal test with factor IX concentrate in the dilution of 1:100 was determined. A positive result of a $10 \times 12 \text{ mm}^2$ wheal was found while a negative control of normal saline showed a $3 \times 4 \text{ mm}^2$ wheal. IgE-mediated anaphylactic reaction was therefore concluded.

The factor IX desensitization protocol was modified from Dioun *et al.* [9] (Table 1) but the cumulative dose was aimed at 40 U/kg. On the first day of desensitization,

the patient developed urticarial rash, tightness of breathing and decreased peak expiratory flow rate at a dose of 0.4 U/kg with a cumulative dose of 0.85 U/kg. The desensitization was stopped and intravenous chlorpheniramine was given. Two days later, the desensitization was restarted. The patient could tolerate a dose of 10.0 U/kg with a cumulative dose of 40.75 U/kg. After that, 40 U/kg factor IX concentrate was successfully given through intravenous infusion, and finally a slow intravenous push.

The daily inhibitor was closely monitored during the desensitization process. On day 6 the inhibitor was 16 BU, and this abruptly increased to 180 BU on day 9. Rituximab 375 mg/m² per week was therefore initiated. Daily factor IX concentrate at the dose of 40 U/kg was continuously given. Four doses of rituximab were administered. After the second dose of rituximab, the CD19-positive and CD20-positive B cells were markedly decreased. The inhibitor gradually decreased to 21.5 BU after the fourth dose of rituximab. Daily 40 U/kg factor IX concentrate was given for 1 month and then decreased to three times per week for another month, to twice per week for 5 months and then to once per week for the remaining 5 months of the first year of ITT. After the evaluation of inhibitor levels and bleeding episodes that were effectively controlled by recombinant activated factor VII combined with local measures during the first year of ITT (shown in Fig. 1 and Table 2), factor IX concentrate was increased to twice per week starting from the second year of ITT. The inhibitor at the 16th month was 10.0 BU.

Two episodes of adverse reaction to factor IX concentrate administration occurred at the fourth month of ITT. The first episode was erythematous rash immediately after receiving factor IX concentrate, which was responsive to intravenous hydrocortisone. Three days later, the patient developed urticarial rash after receiving factor IX concentrate for 15 min that was responsive to intravenous chlorpheniramine administration. Therefore, 10 mg cetirizine was given at bed time and intravenous chlorpheniramine was given before the factor IX concentrate administration for 1 week. After that, only cetirizine was taken at bed time. The patient could tolerate factor IX concentrate administration properly, and cetirizine was discontinued after 1 month. No adverse allergic reaction was detected. At the 15th month of ITT, the intradermal test with factor IX concentrate at the dilution of 1:100 was repeated and revealed positive results.

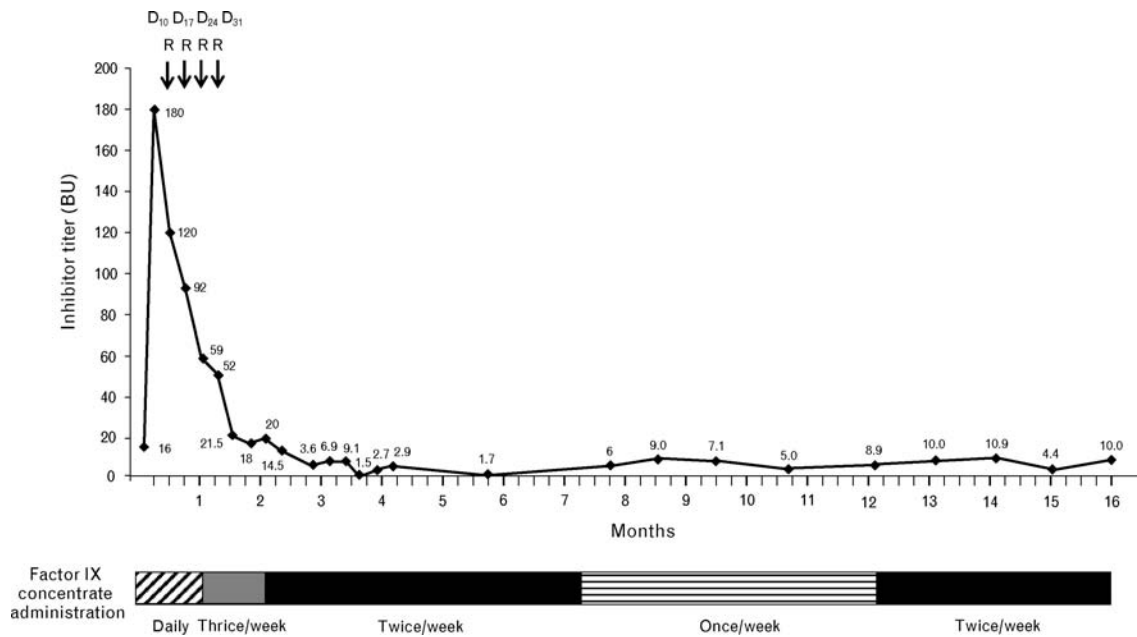
The immunoglobulin levels of IgG, IgM and IgA were monitored monthly. All were within the normal range. Also, urine examination was taken and renal functions were closely monitored. No proteinuria or alteration of renal function was found. The patient attended regular school starting from the third month of ITT and received

Table 1 Factor IX desensitization protocol^a

	Dose (U/kg)	Cumulative dose (U/kg)	Method	Interval from infusion of previous dose (min)
Day 1	0.01	0.01	Slow intravenous push	0
	0.02	0.03	Slow intravenous push	10
	0.04	0.07	Slow intravenous push	10
	0.08	0.15	Slow intravenous push	10
	0.10	0.25	Slow intravenous push	10
	0.2	0.45	Slow intravenous push	20
	0.4	0.85	Slow intravenous push	20
	0.6	1.45	Slow intravenous push	20
	0.8	2.25	Slow intravenous push	20
	1.0	3.25	Slow intravenous push	20
	1.5	4.75	Slow intravenous push	20
	3.0	7.75	Continues infusion over 30 min	–
	6.0	13.15	Continues infusion over 30 min	–
	8.0	21.75	Continues infusion over 30 min	–
	9.0	30.75	Continues infusion over 60 min	–
	10.0	40.75	Continues infusion over 60 min	–
Day 2	40	40	Continues infusion over 10 h	–
Day 3	40	40	Continues infusion over 8 h	–
Day 4	40	40	Continues infusion over 6 h	–
Day 5	40	40	Continues infusion over 4 h	–
Day 6	40	40	Continues infusion over 2 h	–
Day 7	40	40	Continues infusion over 1 h	–
Day 8	40	40	Continues infusion over 30 min	–
Day 9	40	40	Slow intravenous push	–

^a Modified from Dioun *et al.* [9].

Fig. 1



The inhibitor titer during immune tolerance therapy combined with rituximab (R) administration. BU, Bethesda units.

factor IX concentrate at a well equipped in-patient ward setting with the facility to manage the emergency situations during the first year of ITT. Finally, the factor IX concentrate was administered at the out-patient clinic starting from the second year of ITT.

Discussion

Nowadays, more than 30 patients with anaphylaxis to factor IX concentrate have been reported worldwide.

Table 2 Frequencies of bleeding episodes in relation to factor IX concentrate administration (40 U/kg) during the immune tolerance therapy (ITT)

Duration of ITT (months)	Inhibitor (Bethesda units)	Factor IX concentration administration	Bleeding episode
1	16.0–180.0	Daily	Right elbow hemarthrosis
2	28.0–59.0	Thrice/week	Left elbow hemarthrosis
3	3.6–20.0	Twice/week	Right elbow hemarthrosis
4–6	1.7–9.1	Twice/week	–
7	1.7–6.0	Once/week	Right elbow hemarthrosis
8	6.0	Once/week	Left knee hemarthrosis Bleeding at gum and teeth requiring two tooth extractions
9	7.1	Once/week	Right elbow hemarthrosis
10	5.0	Once/week	–
11	5.0	Once/week	Left thigh hematoma
12	8.9	Once/week	–
13	10.0	Twice/week	Ileopsoas muscle bleeding
14	10.9	Twice/week	Gluteal muscle bleeding, right elbow hemarthrosis
15	4.4	Twice/week	–
16	10.0	Twice/week	–

The precise mechanisms of such adverse reactions remain unclear. Sawamoto *et al.* [10] have shown the presence of a specific IgG₁ subclass of antibody to factor IX at the time of anaphylactic reaction in addition to the more common IgG₄ subclass, and have postulated that complement activation may be a contributing factor. On the other hand, Dioun *et al.* [9] demonstrated that these reactions were mediated by IgE, which was also found in our patient revealing positive intradermal testing.

The coexisting inhibitor and anaphylaxis in patients with hemophilia B create constraints in patient management. Moreover, the complication of nephrotic syndrome developed after 8–9 months of ITT is another constraint [9,11,12]. Although the pathogenesis of nephrotic syndrome is not well established, high antigen exposure may be one of the contributing factors. The target dose of factor IX concentrate in the present study therefore aimed at the intermediate dose of 40 U/kg. Also, anti-CD20-positive B-cell monoclonal antibody of rituximab was included as an adjuvant for ITT in this study. Even though a previous study reported the failure of rituximab in ITT in a boy with hemophilia B [8], their patient did not receive factor IX concentrate until 3 months after rituximab administration. In our reported patient, factor IX concentrate was simultaneously administered during the rituximab treatment. The CD20-positive B cells producing inhibitor would be gradually destroyed by mean of the anti-CD20-positive B-cell monoclonal antibody.

The frequency of bleeding episodes during the ITT period in this reported patient was not as low as those of the previously reported patients with hemophilia A at 2 years of age [7,8]. The reason for this finding is that our reported patient was 10 years of age with existing targeted hemophilic arthropathies at both the elbow and knee joints. The frequency of bleeding episodes, however, was decreased after factor IX concentrate was given twice per week. Long-term follow-up was warranted.

As a result, an intermediate dose of 40 U/kg factor IX concentrate was properly tolerated by the desensitization in a hemophilia B boy with anaphylaxis to factor IX concentrate. The subsequent occurrence of inhibitor was reduced by ITT. Rituximab is a helpful adjuvant to ITT, and therefore simultaneous administration of factor IX concentrate and rituximab is emphasized. Essentially, the risk of allergic reaction during ITT should be closely monitored in a well equipped hospital or clinical setting to manage such emergency situations.

References

- 1 Lusher JM, Arkin S, Abildaard CF, Schwartz RS. Recombinant FVIII in previously untreated subjects with hemophilia A: a 3.5 year observational study of safety, efficacy and inhibitor development. *N Engl J Med* 1993; **328**:453–459.
- 2 Ehrenforth S, Kreuz W, Scharrer I, Linde R, Funk M, Gungor T, *et al.* Incidence of development of factor VIII and factor IX inhibitors in haemophiliacs. *Lancet* 1992; **339**:594–598.
- 3 Briet E. Factor IX inhibitors in hemophilia B patients: their incidence and prospects for development with high purity factor IX products. *Blood Coagul Fibrinolysis* 1991; **2** (Suppl 1):47–50.
- 4 Ljung RC. Gene mutations and inhibitor formation in patients with haemophilia B. *Acta Haematol* 1995; **94** (Suppl 1):49–52.
- 5 Giannelli F, Green PM, High KA, Sommer S, Poon MC, Ludwig M, *et al.* Haemophilia B: database of point mutations and short additions and deletions. *Nucleic Acids Res* 1993; **21**:3075–3087.
- 6 Moschovi M, Aronis S, Trimis G, Platokouki H, Salavoura K, Tzortzatou-Stathopoulou F. Rituximab in the treatment of high responding inhibitors in severe hemophilia A. *Haemophilia* 2006; **12**:95–99.
- 7 Chuansumrit A, Husapadol S, Wongwerawattanakoon P, Hongeng S, Sirachainan N, Pakakasama S. Rituximab as an adjuvant therapy to immune tolerance in a hemophilia A boy with high inhibitor titer. *Haemophilia* 2007; **13**:108–110.
- 8 Mathia M, Khair K, Hann I, Leisner R. Rituximab in the treatment of alloimmune factor VIII and IX antibodies in two children with haemophilia. *Br J Haematol* 2004; **125**:366–368.
- 9 Dioun AF, Ewenstein BM, Geha RS, Schneider LC. Ig E mediated allergy and desensitization to factor IX in hemophilia B. *J Allergy Clin Immunol* 1998; **102**:113–117.
- 10 Sawamoto Y, Shima M, Yamamoto M, Kamisue S, Nakai H, Tanaka I, *et al.* Measurement of antifactor IX Ig G subclasses in haemophilia B patients who developed inhibitors with episodes of allergic reactions to factor IX concentrates. *Thromb Res* 1996; **83**:279–286.
- 11 Barnes C, Brewin T, Ekert H. Induction of immune tolerance and suppression of anaphylaxis in a child with haemophilia B by simple plasmapheresis and antigen exposure: progress report. *Haemophilia* 2001; **7**:439–440.
- 12 Shibata M, Shima M, Misu H, Okimoto Y, Giddings JC, Yoshioka A. Management of haemophilia B inhibitor patients with anaphylactic reaction to FIX concentrate. *Haemophilia* 2003; **9**:269–271.