The Efficacy and Safety of Lyophilized Cryoprecipitate in Hemophilia A

AMPAIWAN CHUANSUMRIT, M.D.*,
AROONRAT CHANTANAKAJORNFUNG, B.Sc.**,
PAKAIMAS PINTADIT, B.Sc.*,
PHONGJAN HATHIRAT, M.D.*,
PARTTRAPORN ISARANGKURA, M.D., D.Sc.*,
KESANEE KUHATHONG, B.Sc.*,
CHATTAYA JITPRAPHAI, M.D.*,
CHAIVEJ NUCHPRAYOON, M.D.**

Abstract

This prospective study of assessing the efficacy and safety of lyophilized cryoprecipitate (LC), which was heat-treated at 60°C for 25 hours, was conducted in 23 patients with hemophilia A (severe 13, moderate 9, mild 1) at the International Hemophilia Training Center, Bangkok from 1997 to 1998. A total of 223 infusions of LC were given. The status of the patients could be classified into 4 groups : group I, non-bleeding (n = 13); group II, severe bleeding requiring hospitalization (n = 9); group III, appendectomy (n = 1) and group IV, early bleeding controlled by modified home treatment (n = 200). Pharmacokinetic studies were conducted in groups I and II. The mean *in vivo* half-life of factor VIII clotting activity (F VIII:C) was 12.6 hours and the mean *in vivo* incremental recovery at baseline was 2.1 per cent/unit/kg. The mean clearance was 3.22 ml/kg/h. There was no statistically significant difference in these parameters between groups I and II ($p > 0.05$). The hemostasis was successfully achieved and 1 to 2 small urticarial wheals were observed in only 2 infusions. In addition, 9 out of 23 patients received LC exclusively for 1 year. None of them developed inhibitor to F VIII:C nor did any contract additional transfusion-transmitted infection except one who developed anti-hepatitis C virus seroconversion after receiving 16 bottles of LC in 4 months. Therefore, the more efficient virus-inactivation in the preparation of LC should be established.

Key word : Lyophilized Cryoprecipitate, Hemophilia, Efficacy, Safety

* International Hemophilia Training Center-Bangkok, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400,
** National Blood Center, Thai Red Cross Society, Bangkok 10330, Thailand.

In Thailand, less than 5 per cent of the hemophiliac patients who received single-unit preparation of non-virus-inactivated cryoprecipitate, fresh frozen plasma or fresh dry plasma contracted human immunodeficiency virus (HIV) infection[1]. Although the laboratory screenings of various infectious markers such as VDRL, hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (HCV), anti-HIV, HIV p24 antigen in every unit of donated blood as well as donor self-exclusion are routinely practiced in the National Blood Center and hospital blood banks in Thailand, the locally-prepared virus-inactivated blood component is obviously desirable for all blood recipients. Recently, the National Blood Center and the Thai Red Cross Society has successfully produced lyophilized cryoprecipitate (LC) which is heat-treated at 60°C for 25 hours. The prospective study of assessing the efficacy and safety of this new blood component was conducted at the International Hemophilia Training Center (IHTC), Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand from 1997 to 1998.

MATERIAL AND METHOD

Patients and samples

The study was approved by the Ethical Committee of the Faculty of Medicine, Ramathibodi Hospital. Informed consents were obtained from 23 parents or patients with hemophilia A (severe = 13, moderate = 9, mild =1) who were unable to afford the imported virus-inactivated factor concentrate. They had no inhibitor to factor VIII clotting activity (F VIII:C). Twenty-one patients have received non-virus-inactivated frozen cryoprecipitate, fresh frozen plasma (FFP) and/or fresh dry plasma (FDP). Only 2 patients with moderate or mild degree were previously-untreated patients.

To obtain FVIII decay curves, patients who had abstained from treatment for 3 days received a single dose of 20-60 units/kg over a period of 30 minutes. Venous blood samples were taken before and at 1, 2, 4, 6, 10-12, 15-18, 21 and 24 hours after the end of infusion. The citrated plasma samples were kept in a -60°C refrigerator until testing. The F VIII:C was measured by the one-stage technique[2] by the ACL automated machine. The factor VIII deficient plasma from Stago® was used as substrate.

Nine out of 23 patients received LC exclusively for a period of 1 year. Venous blood samples were taken before and at 1, 3, 6, 9 and 12 months after commencing this program for inhibitors to FVIII:C[3], HBs Ag, anti-HCV, anti-HIV, HIV p24 antigen, a liver profile and other biochemistry parameters.

Source of factor VIII

Fifteen bags of cryoprecipitate were pooled under the laminar air flow and divided into 5 separated bottles. Then, they were lyophilized and subsequently heat-treated at 60°C for 25 hours by the modified method of Skjonsberg et al[4,5]. The heat-treated LC achieved the quality control testings of toxicity, pyrogen and sterility. Each bottle of LC was dissolved with 50 ml of sterile water to obtain the clear yellow plasma containing approximately 300 units of FVIII:C. Then, one millilitre of dissolved plasma from each bottle of LC, which was infused to the patient through a blood transfusion set, was kept in a -60°C refrigerator and assayed for the F VIII: C levels.

Pharmocokinetic calculation

The computerized calculations have been previously described[6]. In brief, the exponential function $C_{(t)} = A.e^{-kt}$ was fitted to the FVIII:C *vs* time data obtained after the single dose of FVIII. $C_{(t)}$ is the 'concentration' of FVIII:C as a function of time, A is the zero time intercept of the curve and k is the elimination rate constant. The elimination half-life is (ln 2)/k. In addition, the clearance at a steady state of FVIII:C was calculated by standard 'model-independent' procedures[7-10].

Statistics

The comparison between the two groups of patients was calculated by Mann-Whitney U-Wilcoxan Rank Sum W Test. A p value of less than 0.05 was considered significant.

RESULTS

Twenty-three patients, whose ages ranged from 1 to 39 years with the mean age of 17 years and 8 months, enrolled in the study. The body weight ranged from 10.8 to 67 kg with a mean weight of 43 kg. A total of 223 infusions of 2 to 5 bottles of LC were given. The status of the patients could be classified into 4 groups : group I, non-bleeding (n = 13); group II, severe bleeding requiring hospitalization (n = 9); group III, appendectomy (n = 1) and group IV, early bleeding controlled by modified home treatment (n = 200). The severe

bleeding episodes included punching injuries directly to the eye inducing chemosis of conjunctiva and periorbital hematoma, 3 cases; hemarthrosis, 3 cases; massive gross hematuria, large hematoma at the back and hematoma at the forehead, one each. The pharmacokinetic studies were conducted in group I and group II. The mean *in vivo* half-life of F VIII:C was 12.6 hours and the mean *in vivo* incremental recovery at baseline was 2.1 per cent per unit per kilogram. The mean clearance was 3.22 ml/kg/h. There was no statistically significant difference in these parameters between group I and group II ($p > 0.05$). The F VIII:C levels were appropriately raised according to the bleeding symptom and procedure. The response to LC was categorized as good or excellent, the hemostasis was successfully achieved and the surgery was uneventfully performed. The adverse reactions were observed in only 2 infusions which were 1 to 2 small urticarial wheals.

Nine out of 23 patients (39%) received LC exclusively for all bleeding episodes and modified home treatment for a period of 1 year. The descriptive data was shown in Table 1. All of them were negative for HBsAg, anti-HIV and HIV p24 Ag. Five cases received hepatitis B vaccine and 4 cases were positive for anti-HCV and anti-HBs. However, their liver profiles and other biochemistry parameters were normal. Their ages ranged from 1 to 14 years with the mean age of 9 years and 3 months. The body weight ranged from 10.8 to 67 kg with the median weight of 32 kg. Regarding the modified home treatment, the parents purchased LC from the IHTC in Bangkok, transported them in an ice box and kept them in a 4°C home refrigerator. In cases of early bleeding episodes, the parents brought the patients to see the medical personnel at the health stations, district hospitals or Ramathibodi Hospital for 1-3 bottles of LC infusion according to the prescription of the hematologist in IHTC, Bangkok. During the 1 year follow-up, the occurrence of bleeding ranged from 5 to 44 episodes and 13 to 99 bottles of LC were transfused. Neither of 9 patients developed inhibitors to F VIII: C nor contracted additional transfusion-transmitted infection except one who developed anti-HCV seroconversion after receiving 16 bottles of LC in the period of 4 months. However, there was no alteration in the liver profiles and other biochemistry parameters.

Table 1. Descriptive data of hemophiliac patients receiving lyophilized cryoprecipitate exclusively for a period of one year.

			Blood component received before 1997				Bleeding in 1997-1998		Infectious markers before and after enrolling in the study			
									HBsAg, anti-HIV, HIV Ag		Anti – HCV	
Age (yr)	FVIII:C (%)	BW (kg)	Frozen cryoppt. (bags)	FFP (ml)	FDP (bottles)	Factor conc. (units)	Episodes	Lyophilized cryoppt. (bottles)	Before	After	Before	After
14	< 1	67	339	1,450	183	–	20	99	–	–	–	–
14	< 1	36	2,249	8,310	155	–	22	44	–	–	+	+
12	< 1	45	263	600	138	–	21	41	–	–	+	+
12	3	32	344	–	99	–	26	26	–	–	+	+
10	1	20	246	200	47	15,920	21	21	–	–	+	+
8	< 1	30	459	420	–	1,000	5	13	–	–	–	–
3	2	15	–	–	10	–	19	19	–	–	–	–
1	3	11	30	–	–	–	22	22	–	–	–	+ *
1	1	12	–	–	–	–	44	44	–	–	–	–

* After enrolling in the study for 4 months

DISCUSSION

The developing countries contain 3 quarters of the world's population. Most of the hemophiliacs in the developing countries receive inadequate replacement therapy or only non-virus-inactivated blood components of FFP, cryoprecipitate or FDP[11]. Since the replacement therapy is inevitably avoided in the bleeding episodes, the safe virus-inactivated blood component is required. In this study, the mean *in vivo* half life of F VIII:C of LC did not differ from those of FDP, FFP, cryoprecipitate, or factor concentrate[12]. However, the *in vivo* incremental recovery at baseline was higher than those of FFP, cryoprecipitate or FDP[13] and was similar to the intermediate purity factor concentrate. The clearance was also similar to the intermediate purity factor concentrate. The efficacy of LC was clearly shown in the study. The hemostasis was successfully achieved, the bleeding was controlled and the surgery was uneventfully performed. On the contrary, the inconvenience of LC was the higher volume of 50 ml as compared to 10 ml of factor concentrate in 250 units of F VIII:C. However, it can be easily given *via* a blood transfusion set. The larger volume was outweighed by the lower price. The LC of 300 units F VIII:C costs 500 baht while the factor concentrate of 250 units costs 3,000 baht.

Before enrolling in the study, 4 patients were positive for anti-HCV since they have received anti-HCV unscreened blood components before 1991. However, the virus-inactivation at 60°C for 25 hours is not an optimal method for eradicating transfusion-transmitted diseases, 1 patient developed anti-HCV seroconversion after receiving 16 bottles of LC in 4 months. He also received 30 bags of frozen cryoprecipitate before enrolling in the study. In spite of screening for anti-HCV by using the second generation of ELISA test in every unit of blood, the hepatitis C virus can possibly be transmitted by either frozen cryoprecipitate[14] or lyophilized cryoprecipitate. The hepatitis C infection transmitted by dry heat-treated factor concentrate at 60-68°C for 24-72 hours among the hemophiliacs has been reported[15].

Therefore, the more efficient virus-inactivation at 80°C for 72 hours or solvent-detergent treated should be established. In addition, if the recombinant factor concentrate can be sufficiently produced to serve the needs of people with hemophilia around the world, it would be an ideal solution for safe and adequate replacement therapy.

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ประสิทธิภาพและความปลอดภัยของไครโอปรีซิปิเตทชนิดผงในผู้ป่วยโรคฮีโมฟีเลีย

อำไพวรรณ จวนสัมฤทธิ์, พ.บ., ภัทรพร อิศรางกูร ณ อยุธยา, พ.บ.*,*
*อรุณรัตน์ จันทนขจรฟุ้ง, ภ.บ.**, เกษณี คูหาทอง, วท.บ.*,*
ผกายมาศ ปิณฑะดิษ, วท.บ., ฉัฐยา จิตประไพ, พ.บ.*,*
พงษ์จันทร์ หัตถีรัตน์, พ.บ., ชัยเวช นุชประยูร, พ.บ.***

การศึกษาประสิทธิภาพและความปลอดภัยของไครโอปรีซิปิเตทชนิดผงที่ผ่านความร้อน 60°ซ นาน 25 ชั่วโมง ผลิตโดยศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย ในผู้ป่วยโรคฮีโมฟีเลีย เอ จำนวน 23 ราย (ชนิดรุนแรงมาก 13, ปานกลาง 9, น้อย 1) จำนวน 223 ครั้ง ที่คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี ในระหว่างพ.ศ.2540 ถึง 2541 แบ่งผู้ป่วยเป็น 4 กลุ่มคือ กลุ่มที่ 1 ผู้ป่วยไม่มีอาการเลือดออก (n = 13) กลุ่มที่ 2 ผู้ป่วยที่มีอาการเลือดออกมากที่ต้องรับไว้รักษาในโรงพยาบาล (n = 9) กลุ่มที่ 3 ผู้ป่วยได้รับการผ่าตัดไส้ติ่ง (n = 1) และกลุ่มที่ 4 ผู้ป่วยที่เริ่มมีอาการเลือดออกที่ได้รับการรักษาที่บ้าน (n = 200) คณะผู้รายงานได้ศึกษาเภสัชจลนศาสตร์ในผู้ป่วยกลุ่มที่ 1 และ 2 ปรากฏว่าค่าเฉลี่ย half life ของ factor VIII clotting activity (F VIII:C) เท่ากับ 12.6 ชั่วโมง สามารถเพิ่มระดับ F VIII:C ในผู้ป่วยได้เท่ากับ 2.1%/ยูนิต/กก. และมีค่าเฉลี่ย clearance เท่ากับ 3.2 มล./กก./ชม. โดยที่ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติระหว่างผู้ป่วยกลุ่มที่ 1 และกลุ่มที่ 2 ไครโอปรีซิปิเตทชนิดผงสามารถหยุดอาการเลือดออกได้ดี และผลแทรกซ้อนมีเพียงผื่นลมพิษ 1-2 ตำแหน่งในการให้ไครโอปรีซิปิเตทชนิดผง 2 ครั้งเท่านั้น ผู้ป่วย 9 รายได้รับไครโอปรีซิปิเตทชนิดผงเพื่อรักษาภาวะเลือดออกติดต่อกันเป็นเวลาหนึ่งปี ปรากฏว่าไม่มีผู้ป่วยรายใดเกิดสารต้านแฟคเตอร์ VIII รวมทั้งไม่ได้รับโรคติดเชื้อจากการรับเลือดเพิ่มขึ้น ยกเว้นผู้ป่วยหนึ่งรายมี anti-HCV seroconversion หลังได้รับไครโอปรีซิปิเตทชนิดผงจำนวน 16 ขวดในระยะเวลา 4 เดือน ดังนั้นในการเตรียมส่วนประกอบของเลือดควรจะพัฒนาวิธีฆ่าเชื้อโรคให้มีประสิทธิผลดียิ่งขึ้น

คำสำคัญ : ไครโอปรีซิปิเตทชนิดผง, โรคฮีโมฟีเลีย, ประสิทธิภาพ, ความปลอดภัย

* International Hemophilia Training Center, คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10400
** ศูนย์บริการโลหิตแห่งชาติ, สภากาชาดไทย, กรุงเทพ ฯ 10330