

Influence of heat treatment on FVIII:C recovery from freeze dried cryoprecipitate

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SUMMARY A standard lyophilised triple cryoprecipitate preparation, stabilised by the addition of Synthamin 17, was heat treated at 60°C for 48 hours. The total protein content, factor VIII concentration, and factor VIII recovery were not affected by the heat treatment procedure. Heat treatment did not influence the reconstitution characteristics of the freeze dried preparation and there were no side effects during or after administration. The mean *in vivo* rise of factor VIII from infused heat treated triple cryoprecipitate was 2.5 (SD 0.9) %/unit/kg with a half life of 13.1 (3.1) hours. These results compare favourably with those obtained using non-heated triple cryoprecipitate. Cryoprecipitate can be heat treated without adversely influencing factor VIII recovery, and the ability to prepare a heat treated cryoprecipitate means that a small pool high yield factor VIII preparation can again be used in routine clinical practice.

The heat treatment of large pool factor VIII concentrates is universally recommended for the inactivation of the human immunodeficiency virus (HIV).¹ Although effective against HIV, most heat treatment procedures fail to inactivate completely hepatitis viruses which can be present in blood products prepared from large plasma pools.²

Cryoprecipitate has been widely used for the management of patients with an occasional requirement for factor VIII replacement or in young, newly diagnosed haemophiliacs.³ This product can be prepared by any modestly equipped blood products laboratory. The material also has the considerable production advantages of a relatively higher yield of factor VIII and a small production pool with a correspondingly reduced risk of contamination with hepatitis virus.^{4,5} Confirmed HIV infection treated with non-heated cryoprecipitate means, however, that this product is no longer acceptable for the routine treatment of patients with haemophilia.⁶

The high concentrations of fibrinogen and other labile plasma proteins have previously caused practical difficulties in preparing heat treated cryoprecipitate preparations. *In vitro* studies using amino acid mixtures to stabilise the proteins in cryoprecipitate during heat treatment have now been reported.⁷ The addition of the amino acid mixture Synthamin 17 has enhanced stability of

cryoprecipitate during heat treatment. We now report favourable *in vitro* recovery of factor VIII following the infusion of heat treated cryoprecipitate into patients with classic haemophilia.

Material and methods

Blood (430 ml) was collected from random, healthy donors into 63 ml citrate-phosphate-dextrose (CPD) anticoagulant in the primary bag of a triple blood pack (Tuta Laboratories, Australia). The cryoprecipitate was recovered as previously described⁸ and, after the addition of 15 ml of Synthamin 17-glycine buffer (Synthamin 0.38% w/v, 0.08 M glycine, 0.011 M sodium citrate, pH 6.1), aseptically injected into each bag using a disposable dispenser (Pharm-Aide, American Pharmaseal Laboratories, USA). Three bags were connected to a 120 ml MRC plasma bottle using a transfer set (Codan BC 333, Portugal) and the cryoprecipitate in the bags resolubilised by briefly immersing the lower 60 mm of each bag in a 30°C water bath. The cryoprecipitates were pooled by gravity transfer and immediately frozen at -30°C.

The product was freeze dried in a Virtis 250 SRC freeze drier at an initial shelf temperature of 30°C and a maximum product temperature of 15°C. The bottles were closed under vacuum in the freeze drier.

The freeze dried product was heat treated in a hot air oven at 60°C for 48 hours before reconstituting with 40 ml of sterile water for intravenous administration.

Factor VIII assays of both the concentrate and patient plasmas were performed using a standard one stage manual assay and an artificial substrate.⁹ The plasma standard was calibrated against the second WHO international reference plasma. The coefficient of variation was $\pm 13\%$. Von Willebrand factor (VWF) multimeric analysis on the heated and unheated cryoprecipitate preparations was performed as previously described.¹⁰ Fibrinogen was assayed using a spectrophotometric modification of a standard method.¹¹ Total protein was estimated using a modified Biuret method.¹²

Fourteen patients with severe or moderately severe classic haemophilia, who were not actively bleeding, participated in the two recovery trials using the non-heated and heat treated cryoprecipitate. No patient has received blood products during the preceding four days. The product was administered as a single intravenous infusion over 20 minutes through a butterfly needle inserted into a peripheral vein. The age range of the recipients was 7–45 years and body weight varied between 25–80 kg.

In vitro factor VIII concentrations were measured by separate venipuncture before and immediately after infusion (30–45 minutes). In the six patients in whom the half life ($T_{1/2}$) was determined, additional samples were taken at six and 24 hours after administration. Factor VIII recovery (the K value) was calculated from the product of the observed increment in plasma factor VIII and the plasma volume, relative to the expected increase based on the number of factor VIII units administered. For the recovery studies the plasma volume determination was based on body weight calculation using 42 ml/kg with an haematocrit correction.¹³ The half life ($T_{1/2}$) was calculated from the factor VIII survival curve as described previously.¹⁴ The trial was approved by the medical ethical committee, Auckland Hospital, Auckland, New Zealand.

Results

Forty eight bottles of unheated cryoprecipitate and 16 bottles of cryoprecipitate, sampled after heat treatment, were randomly selected for comparative analysis of protein content and reconstitution characteristics (table 1). The reconstitution time, factor VIII potency, yield from starting plasma and VWF multimeric composition (data not shown) were not significantly different after heat exposure. The reconstituted solution was available for infusion within 10 minutes of addition of the sterile water and did not contain visible particulate matter. A filter (Alpha Micron-40, Alpha Therapeutic Corporation, California, USA) was used to draw the material into the syringe.

There were no adverse local or systemic reactions

Table 1 Comparison of cryoprecipitate before and after heat treatment (minutes)

	Before heat treatment	After heat treatment
Factor VIII C (U/ml)	7.3	6.0
Fibrinogen (mg/ml)	18.8	9.0
Total protein (mg/ml)	25.1	32.0
Factor VIII specific activity (U/mg)	0.3	0.2
Reconstitution time (SD)	8 (1.5)	7 (1.2)
pH after recovery	7.2	7.3
Citrate concentration (mM/L)	15	11
Factor VIII recovery (U/kg plasma)	414	367

Table 2 Mean (SD) dosage, recovery, and half life determinations for unheated and heat treated cryoprecipitates

Patients	Dose (units/kg)	Per cent rise/u/kg	Recovery (K)	Half life (hours)
Before heat (n = 6)	12.6	1.94 (0.7)	1.6 (0.2)	12.0 (1.75)
After heat (n = 14)	15.8	2.5 (0.9)	1.1 (0.5)	13.1 (3.1)*

*The half life calculation was determined on the data derived from six patients.

during or after the intravenous infusion. The in vitro recovery, expressed as the mean percentage rise/unit/kg of factor VIII infused, the mean recoveries, and mean half life determinations after administration of the two cryoprecipitate preparations is shown in table 2. The six recipients of the untreated material participated in both trials.

Discussion

In a recent study, using a controlled pore glass high purity factor VIII concentrate, we confirmed the ability of the amino acid mixture Synthamin 17 to stabilise factor VIII following heat treatment at 60°C for 72 hours.¹⁵ After the addition of Synthamin 17, heat treatment of the freeze dried concentrate resulted in less than 10% further loss of factor VIII activity, with a final 25% yield of factor VIII from the starting plasma. Infusion of this stabilised, and heat treated concentrate into haemophilic patients resulted in excellent in vitro factor VIII recovery.

The amino acid mixture Synthamin 17 also seems to confer stability to heat treatment on a relatively crude cryoprecipitate preparation.⁶ This study now provides further evidence that the heat treatment procedure under these conditions does not adversely influence the solubility or reconstitution of the cryoprecipitate. There were no unexpected side effects during infusion and the in vitro recovery of the infused factor VIII was not significantly different from that observed with unheated cryoprecipitate.

The protective effects of a lowered pH on the stability of cryoprecipitate have recently been shown¹⁶ and have been suggested as an alternative to the

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addition of stabilisers such as Synthamin 17 in the preparation of heat inactivated cryoprecipitate. The report concluded that there were considerable disadvantages with Synthamin 17, which included increased residual moisture resulting in discolouration of the lyophilised material, the potential for a stabilising effect of the amino acids on the virus, and an increase in production costs. In our experience careful monitoring of the freeze drying procedure minimises potential discolouration due to a high residual moisture content. In addition, some residual moisture in the freeze dried preparation may be necessary for effective inactivation of HIV virus (Foster P, personal communication). The added benefit of using Synthamin 17 stabilisation is its relative simplicity as a minor modification of an existing successful cryoprecipitate preparation technique and this greatly outweighs any possible disadvantages attributable to an increase in cost. The considerable buffering capacity of Synthamin 17 also reduces interbatch variability due to significant fluctuations of the pH following lyophilisation.

The method used in this report is now in routine use in our laboratory for preparing a heat treated cryoprecipitate from a triple donor pool for the management of classic haemophilia. The material has also been used to treat patients with von Willebrand's disease undergoing surgical procedures. We are presently producing 1.6 million factor VIII units every year as heat treated cryoprecipitate.

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