

Freeze dried cryoprecipitate: a clinical evaluation

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SUMMARY Freeze dried cryoprecipitate was used in the treatment of 14 patients with haemophilia A. The *in vivo* recovery was 91.2% which is comparable to that reported from other parts of Europe. The product was efficacious and no adverse effects were reported.

Freeze dried cryoprecipitate is the high yield product of a low technology process and as such may be of value in reducing any possible shortfall in the factor VIII requirements of the haemophilic population of the UK.

Human factor VIII is available in the UK as cryoprecipitate or as freeze dried concentrate prepared by the protein fractionation centres of the National Health Service or from commercial sources. Cryoprecipitate¹ offers a source of factor VIII which is simple and inexpensive to manufacture and has a good yield although there is some variation in potency between units.² The requirements for storage at -20°C is a further disadvantage which precludes the use of cryoprecipitate in the home therapy programmes for haemophiliacs. Freeze dried concentrates offer a convenient, easily stored form of human factor VIII of known potency.³ However, these products require expensive fractionation plant for their production whose yield is low. At present, approximately 50% of factor VIII used for home therapy in this country is from commercial (imported) sources.

The use of a freeze dried preparation of cryoprecipitate⁴ would circumvent the storage problems of conventional cryoprecipitate while retaining that product's advantages of high yield, low cost and simple technology. The results of a clinical evaluation of such a product are described.

Material and methods

Freeze dried cryoprecipitate was prepared as described previously.⁴ The method involved the collection of a donation of blood into a triple plastic blood collecting pack containing citrate phosphate dextrose as anticoagulant. Plasma was separated by centrifugation and the plasma pack was immediately

frozen in an alcohol, solid carbon dioxide mixture, kept at -70°C for a minimum of 20 min.

Cryoprecipitate was prepared by thawing the plasma in a thermostatically controlled water bath at 4°C until only a small amount of ice remained in the pack. The pack was removed from the water bath, centrifuged at 3200 g for 10 min at 4°C and supernatant plasma transferred to remaining satellite pack.

Cryoprecipitate was allowed to liquefy at 22°C and cryoprecipitates from five donations were pooled under sterile conditions. The product was then frozen and lyophilised.

All whole blood donations were tested for the presence of hepatitis B surface antigen by a sensitive radioimmunoassay test (Abbott Ausria II Radioimmunoassay test).

The material was reconstituted in 100 ml sterile water immediately prior to infusion.

Venous blood (9 ml) obtained by clean venepuncture before and 20 minutes after infusion of cryoprecipitate, was added to 1 ml of 0.109 M trisodium citrate. Plasma was separated by centrifugation at 2000 g at 4°C for 15 min. Aliquots of plasma were used immediately for factor VIII C assay or stored at -20°C for factor VIII RAG and factor VIII VWF assay.

Factor VIII C was assayed by a standard one-stage assay technique and factor VIII RAG assay using Laurell rocket technique. The assay of factor VIII VWF was performed by measuring the aggregation of fixed pooled platelets induced by ristocetin.⁵ All assays were performed in duplicate and results expressed as a mean. One-hundred per cent standard assays were obtained from plasma pooled from

Freeze-dried cryoprecipitate characteristics

	Mean \pm SEM (IU/ml)	Range (IU/ml)
Factor VIII C	4.35 \pm 0.21	3.20 - 6.20
Factor VIII RAG	8.29 \pm 0.43	4.87 - 11.42
Factor VIII VWF	6.75 \pm 0.59	2.80 - 10.50

Ratio of factor VIII RAG: factor VIII C = 1.91:1.

Ratio of factor VIII VWF: factor VIII C = 1.55:1.

10 healthy volunteers were calibrated against the WHO reference standard in international units. Plasma volume was calculated from standard nomogram.⁶

Recovery was calculated from:

$$\frac{(\text{Factor VIII}_{\text{post}} - \text{Factor VIII}_{\text{pre}}) \times \text{plasma volume (ml)}}{\text{Factor VIII}_{\text{cryo}} \times \text{volume of cryoprecipitate infused (ml)}} \times 100.$$

$$\times 100.$$

where factor VIII_{pre} was the pretreatment level of factor VIII.

factor VIII_{post} was the pretreatment level of factor VIII.

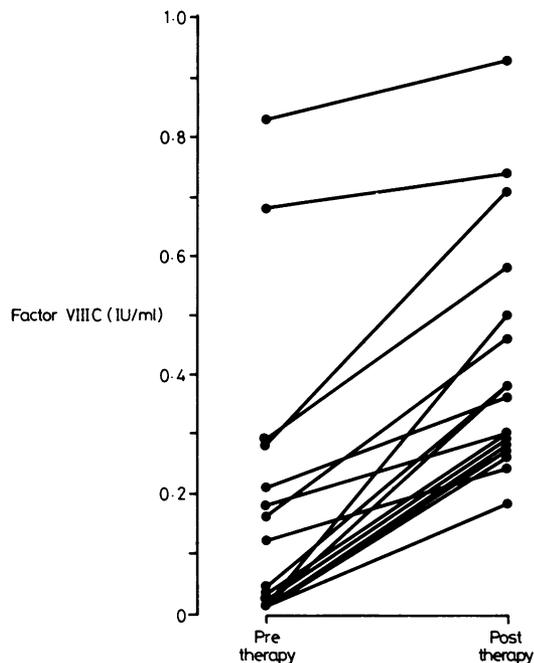
factor VIII_{cryo} was the factor VIII level of cryoprecipitate.

Patients

Fourteen patients with haemophilia A and without inhibitor to factor VIII C were recruited from the Haemophilia Unit of Royal Infirmary, Glasgow. Informed consent was obtained from all patients prior to entry to study. Patients were given a dose of factor VIII (nominally 800 IU of factor VIII C) in the form of freeze-dried cryoprecipitate prior to dental surgery. Two patients each received freeze-dried cryoprecipitate on three occasions. In total, 19 doses of freeze-dried cryoprecipitate were given. The freeze-dried cryoprecipitate was prepared one year prior to the start of the study. In all other respects the patients were managed in accordance with the current practice of the Haemophilia Unit.

Results

The mean (\pm SEM) factor VIII C of the concentrate was 4.35 \pm 0.21 IU/ml. However, there was considerable variation noted: factor VIII C ranged from 3.20–6.20 IU/ml. Similar results were obtained in terms of factor VIII RAG + factor VIII VWF (see Table). Prior to therapy, the mean factor VIII C level was 0.16 IU/ml. Two patients had factor VIII C levels greatly in excess of this (see Figure). Both had received factor VIII concentrate prior to having



Factor VIII C level pre- and postinfusion of freeze-dried cryoprecipitate (860 IU). Mean recovery 91.2% (SEM \pm 11.2%)

freeze dried cryoprecipitate. After 19 infusions of freeze dried cryoprecipitate, the mean factor VIII C rose to 0.40 IU/ml, giving a mean recovery of factor VIII C of 91.2% (see Figure).

Mean plasma volume was 3085 ml.

No adverse effects were reported and no patient required additional factor VIII therapy to control bleeding.

Discussion

The factor VIII C and factor VIII RAG assays of the freeze-dried cryoprecipitate are similar to results obtained elsewhere.^{4,7} The recovery of factor VIII C of 91.2% is slightly lower than the 100% reported from Paris⁷ although difference in the timing of the postinfusion sample may account for this difference.

The low factor VIII RAG: factor VIII C and factor VIII VWF: factor VIII C ratios suggest that little denaturation has taken place during preparation of this product.

The major diadvantage with this product as with conventional cryoprecipitate is the wide variation in factor VIII C from unit to unit. In this study, factor VIII C ranged from 3.20 IU/ml to 6.20 IU/ml. However, the mean factor VIII C actually given (860 IU)

was similar to the factor VIII C nominally present in the 200 ml dose (800 IU).

A minor disadvantage, especially for home therapy use is the volume of the reconstituted product. A dose of 400 IU requires the infusion of 100 ml of reconstituted freeze dried cryoprecipitate whereas with intermediate purity concentrate, 500 IU can be given in 20–30 ml of reconstituted product.

However, the excellent yield and the simple, low cost technology required for its production make this product suitable for further consideration in the UK if the National Health Service protein fractionation centres are unable to meet the demand for factor VIII with the consequent reliance on commercial, imported sources of factor VIII.^{4 8–10} Additionally, freeze dried cryoprecipitate would be particularly suitable for developing countries wishing to provide a haemophiliac therapy programme.

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References

- ¹ Pool JG, Shannon AE. Production of high potency concentrates of antihemophilic globulin in a closed bag system. *N Engl J Med* 1965;**273**:1443–7.
- ² Watt JG. Plasma fractions. In: Cash JD, ed. *Clinics in haematology* London: Saunders, 1976:106–9.
- ³ Jones P, Fearn M, Forbes C, Stuart J. Haemophilia home therapy in the United Kingdom 1975–6. *Br Med J* 1978;*i*:1447–50.
- ⁴ Milligan G, Graham R, Hanratty S, Muir W, Mitchell R. Production of freeze-dried human antihemophilic cryoprecipitate. *J Clin Pathol* 1981;**34**:1091–3.
- ⁵ Giddings JC. Hereditary coagulation disorders: laboratory techniques. In: Thomson JM, ed. *Blood coagulation and haemostasis, a practical guide* 2nd ed. Edinburgh: Churchill-Livingstone 1980:117–57.
- ⁶ Dagher FJ, Lyons JH, Finlayson DC. Blood volume measurement: a critical study prediction of normal values: controlled measurement of sequential changes: choice of a bedside method. *Ad Surg* 1965;**1**:69–109.
- ⁷ Allain JP, Verroust F, Soulier JP. In vitro and in vivo characterisation of Factor VIII preparations. *Vox Sang* 1980;**38**:68–80.
- ⁸ Jones P. Factor VIII supply and demand. *Br Med J* 1980;**281**:1531–2.
- ⁹ Cash JD. Factor VIII supply and demand. *Br Med J* 1980;**281**:565.
- ¹⁰ Gabra GS, Crawford R, Mitchell R. Factor VIII supply and demand. *Br Med J* 1981;**281**:1006.

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