Technical method

Fresh frozen cryosupernatant in place of fresh frozen plasma for broad spectrum coagulation factor replacement

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The use of fresh frozen plasma (FFP) has shown a dramatic increase in recent years. In the Welsh Region, covering a population of 2.2 million, 294 units were supplied in 1974, and this increased to over 5000 in 1983.

Much of the growth in fresh plasma use has been for treatment or prevention of coagulation disorders other than primary factor VIII deficiency or defibrination syndromes. In a recent local survey less than 5% of all FFP issued was required for conditions in which factor VIII values might be expected to be reduced. Most of the FFP was used during plasma exchange, massive transfusion, or cardiac surgery. Previous examination of clotting factor activity in cryosupernatant plasma has shown its potential value as a replacement fluid especially in large volume therapeutic plasma exchange and in severe factor V deficiency.¹⁻³ Despite this evidence, FFP has continued to be the product of choice in a wide variety of conditions. It seemed worthwhile, therefore, to re-examine the potential value of fresh frozen cryosupernatant in place of FFP in circumstances such as fluid replacement for apheresis patients and prophylaxis of coagulation abnormalities occurring during massive transfusion.

Material and methods

Ten units of whole blood from healthy volunteer donors were collected into CPDA-1 triple bags (Travenol PL146). Within 6 h of donation the blood was centrifuged at 3500 g for 10 min at 4°C. The resultant platelet poor plasma was immediately expressed into a 300 ml satellite bag and each unit of concentrated red cells was sealed off. Aliquots of platelet poor plasma were snap frozen in liquid nitrogen for use as reference samples in coagulation

assays. The remainder was frozen for 2 h in metal containers in a blast freezer (Pritchard Laboratory Refrigeration) set at -60°C. The frozen platelet poor plasma was then thawed at 4°C for 12-16 h as for routine cryoprecipitate production. The cryoprecipitate was sedimented by centrifugation at 3500 g for 10 min at 4°C and the excess cryosupernatant was then siphoned into the second satellite bag. The cryoprecipitate, in about 15-20 ml plasma, was redissolved by placing it in a 37°C waterbath for 10 min; aliquots of this and the cryosupernatant were then snap frozen in liquid nitrogen. The volumes of plasma, cryoprecipitate, and cryosupernatant were calculated from their weights.

The coagulation factor content of platelet poor plasma, cryoprecipitate, and cryosupernatant were examined as outlined below:

The kaolin cephalin clotting test (KCCT), one stage prothrombin time, factor V assay, and one stage factor VIII assays were based on the methods described by Austen and Rhymes.⁴ Cephalin and kaolin, however, were replaced by a combined reagent, activated cephaloplastin reagent (Dade), in the KCCT test and one stage factor VIII assay. Brain thromboplastin was obtained from the National UK Reference Laboratory, Manchester; factor V and factor VIII deficient substrate and 100% factor V and factor VIII plasma standard were obtained from Immuno Ltd. The 3rd British Standard for Blood Coagulation Factor VIII Concentrate (human) was supplied by the National Institute for Biological Standards and Control, London. Fibrinogen concentrations were measured using a fibrinogen reagent kit (Boehringer Mannheim) and fibrinogen degradation products by a latex kit (Mercia-Brocades Ltd). Fibronectin was assayed by immunoelectrophoresis using the Laurell rocket technique.⁵ In this technique, 187 µl of rabbit anti-human fibronectin was added to 25 ml of 1% agarose ME Seakem (Miles Laboratories). The electrophoresis buffer was 0.08 M Tris, 0.025 M Tricine buffer, pH 8.6.

Results

The percentages of factor V and factor VIII recovered in the cryosupernatant from the original plasma were 75% and 18.6% respectively (Table). The corresponding figures for fibrinogen and fibronectin were 64.6% and 45.3%.

Measurements of fibrinogen degradation products

Coagulation factor activities of 10 units of platelet poor plasma before and after processing into cryoprecipitate and cryosupernatant plasma

	Platelet poor plasma (Mean 228 ml)	Cryoprecipitate (Mean 17.4 ml)	Cryosupernatant (Mean 210 ml)
Kaolin cephalin clotting time(s) *	51-59 (54.0)	41-51 (44.6)	68-84 (74.0)
One stage prothrombin time(s) *	14-15.5 (14.5)	13.5-16 (15.1)	15-19.6 (17.6)
Fibrinogen degradation products (µg/ml)	<15	<15	<15
Fibrinogen			
% Yield	100.0	32.4	64.6
g/l	2.4	7.8	1.82
g/pack	0.54	0.135	0.382
Range	0.43-0.65	0.1-0.27	0.3-0.47
Factor V			
% Yield	100.0	5.0	75.0
U/ml	0.91	0.66	0.74
U/pack	207.48	11.48	155.4
Range	108-269	5.25-24.3	94.4-191
Factor VIII			
% Yield	100.0	52.6	18.6
U/ml	1.08	7.47	0.21
U/pack	246.20	129.40	44.1
Range	195.6-314.8	76-192	26.9-65
Fibronectin			
% Yield	100.0	49.35	45.3
U/ml	1.41	9.1	0.69
U/pack	321.48	158.3	144.9
Range	173-503.2	90.3-281.6	74.4-241.6

*Values given as range (mean).

were below 15 µg/ml. The mean clotting time of the one stage prothrombin test was 17.6 s and that of the kaolin cephalin clotting test was 74 s.

Discussion

Fresh frozen plasma is a valuable source of material for factor VIII concentrate and its use where factor VIII is not required is wasteful. Cryosupernatant shares many of the features of fresh frozen plasma but is relatively more economical. Despite this, the use of fresh frozen plasma has grown dramatically, while cryosupernatant plasma has been neglected as a possible alternative.

The coagulation factor composition of cryosupernatant as measured in this study shows clearly that even extensive transfusion of such fluid would not result in clinically significant coagulation abnormalities. Most factors are present in adequate concentrations for haemostasis. Factors VII, IX, and X have already been shown not to be removed by the cryoprecipitation process.¹ It is probable that the slightly prolonged KCCT times in this report reflect the deficiency of factor VIII in cryosupernatant. Addition of FVIII in the form of NHS intermediate concentrate almost corrects the KCCT time to normal. The concentration of fibrinogen degradation products is sufficiently low to be clinically insignificant. Frozen cryosupernatant could therefore be substituted for fresh frozen plasma for many of the clinical purposes for which the latter material

is used. Cryoprecipitate, fresh blood, or plasma are best suited for management of the relatively uncommon defibrination syndrome and these products would also be preferred for broad spectrum haemostatic defects in neonates. Fresh frozen plasma should probably continue to be used for treatment of coagulation problems as a result of liver failure or the relatively uncommon congenital coagulation deficiency states for which specific concentrates are not available. Cryosupernatant seems well suited to use in place of fresh frozen plasma during plasma exchange and it has been used instead of albumin for this purpose without adverse effects.⁷ There seems little reason why frozen cryosupernatant should not be substituted for fresh frozen plasma during major blood loss or cardiovascular surgery, especially when it is used to prevent the development of dilutional coagulation factor deficiencies. This wasteful practice of using fresh frozen plasma seems to have gained widespread acceptance even though it has been shown that clinically significant deficiencies are found relatively infrequently.⁹ Prudent practice would entail more frequent use of platelet count and coagulation screening tests to identify those patients with haemostatic defects who may require more energetic replacement treatment.

Fibronectin deficiency is a possible though unproved phenomenon of clinical importance,^{9,10} and preliminary attempts at replacement treatment have been reported.¹¹ Since cryosupernatant contains around 45% of the original fibronectin it seems

possible that some clinically useful replacement could be provided if several units of cryosupernatant plasma were transfused. Clearly, cryoprecipitate would be required to rectify a major deficiency state.

From the data presented above cryoprecipitate supernatant appears to have a therapeutic potential that is currently not fully exploited. This may be of particular importance in areas where shortages of all forms of plasma proteins for transfusion restrict the quality of medical care.

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References

¹ Graybeal FR, Mooreside DE, Langdell RD. Clotting factor activity in cryoprecipitates and supernatant plasma prepared from the blood collected into ACD, ACD-Adenine, CPD and CPD-Adenine and from plasma collected by plasmapheresis. *Transfusion* 1969;9:135-40.

- ² Katz AJ, Morse EE. Factor V activity in fresh frozen and cryoprecipitate removed plasma. *Vox Sang* 1972;22:39-44.
³ Ahr J, Adjizian JC, Droule Ch, *et al*. Cryoprecipitate depleted plasma as a replacement fluid. *Plasma Therapy* 1981;2:243-6.
⁴ Austen DEG, Rhymes IL. *A laboratory manual of blood coagulation*. Oxford: Blackwell Scientific Publications, 1975.
⁵ Laurell CB. Electroimmunoassay. *Scand J Clin Lab Invest*. 1972;29 (suppl 124):21-37.
⁶ Monthey JF, Wallace EG, Allen DM. A non-barbital buffer for immunoelectrophoresis in agarose gels. *Clin Chem* 1978;24:1825-8.
⁷ Das PC, Smit Sibinga C Th. Replacement fluid in plasma exchange. *Lancet* 1980;ii:644.
⁸ Counts RB, Haisch C, Simon TL, *et al*. Haemostasis in massively transfused trauma patients. *Ann Surg* 1979;190:91.
⁹ Anonymous. Fibronectin and infection (Editorial) *Lancet* 1983;i:106-7.
¹⁰ Doran JE. A critical assessment of fibronectin's opsonic role for bacteria and microaggregate. *Vox Sang* 1983;45:337-48.
¹¹ Boughton BJ, Simpson A. Plasma fibronectin in acute leukaemia. *Br J Haematol* 1982;51:487-91.

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Letters to the Editor

Columnar epithelial lined oesophagus (CELO) or Barrett's oesophagus: mucin histochemistry, dysplasia, and invasive adenocarcinoma

We were interested to read the recent article¹ which discussed the incidence and significance of type IIB intestinal metaplasia in biopsies from a series of symptomatic patients with columnar epithelial lined oesophagus (CELO). We have recently studied the mucosal appearances and mucin histochemistry in three patients with endoscopic and histological confirmation of CELO who also had dysplasia. Our findings, together with those of Peuchmaur *et al*¹ and those of Smith *et al*² in another

recent study, strongly support a sequence of dysplasia, in situ carcinoma, invasive carcinoma in patients with CELO.

The clinical and pathological features of our three patients are summarised in the Table. Their ages and symptoms are similar to those of patients in other series,² and in all three patients the endoscopic appearances were non-specific. The entire mucosal surfaces of the two partial oesophagectomy specimens were embedded and examined histologically. Dysplasia in the positive biopsies and resection specimens was graded according to a standard classification,³ which has also been used in other studies.^{2,4} The mucin histochemistry was shown and classified as originally applied to CELO.⁵

There was no evidence of invasive adenocarcinoma in any of the histological material examined from these patients.

Analysis of the constituent mucin histochemistry showed type IIB intestinal metaplasia (sulphomucins in columnar cells) in all three patients.

Although our findings may not be particularly surprising, we believe that they illustrate several points of interest with regard to CELO. Peuchmaur *et al*¹ suggest that type IIB intestinal metaplasia in CELO may represent a premalignant lesion. This is based on the observed high incidence of type IIB metaplasia in CELO, the recognised association between this type of metaplasia and adenocarcinoma of the stomach, and also the recently established premalignant character of CELO. The association between type IIB intestinal metaplasia and high grade dysplasia (including in situ carcinoma) is confirmed in our three patients (Table).

Clinical and pathological features of three patients with columnar epithelial lined oesophagus (CELO)

Patient	Sex	Age	Symptoms (Duration)	Positive biopsy	Previous biopsy	Oesophagectomy	Type of CELO	Course
1	Male	66	Dysphagia (2 years)	High grade dysplasia (in situ carcinoma)	Inflammatory (and ulcers)	Multiple inflammatory ulcers Multifocal high and low grade dysplasia No in situ or invasive carcinoma	IIB	Well, 4 months after operation
2	Male	69	Reflux (2 years)	High grade dysplasia (in situ carcinoma)	Inflammatory	Multifocal high and low grade dysplasia In situ carcinoma No invasive carcinoma	IIB	Died 14 days after operation Necropsy showed bronchopneumonia; no evidence of carcinoma
3	Female	78	Dysphagia (4 years)	High grade dysplasia	No	Medically unfit for surgery	IIB	Some dysphagia; on a semi-solid diet; otherwise well