CONFIDENTIAL

SNBTS FVIII STUDY GROUP

Progress Report on Studies to Improve Yield and Quality of FVIII Concentrate

P. R. Foster
Protein Fractionation Centre
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1. Introduction

These notes provide an up-dating of the previous report (24 January 1983) and should be read in conjunction with that report.

2. Preventing FVIII Inactivation due to Calcium Depletion

Previous studies have linked FVIII inactivation to low concentrations of ionised calcium. This phenomenon is being studied at 3 points in the overall process.

2.1 Plasma (Study at Edinburgh BTS)

The influence of calcium on plasma FVIII:C has been investigated by monitoring plasma collected in:-

(i) CPD

(ii) CPD + Calcium

(iii) Heparin

(iv) Heparin + Citrate

(v) Heparin + Calcium

Initial results (A. Farugia) suggest that the addition of calcium to CPD plasma may help to prevent loss of FVIII:C.

Stability of FVIII:C in plasma of various Ca^{2+} concentrations

Solution	$CaCl_2$ (mM)	Na Citrate (mM)	Ca^{2+} (mM)	% FVIII:C after O/N hold
CPD plasma	0	0	< 0.01	63 ± 14.7
"	10	0	0.07	80.5 ± 14.2
"	15	0	0.33	72·5 ± 8·6
"	20	0	0.44	82.8 ± 9.8
"	25	0	0.96	77.5 ± 10.3
"	30	0	0.78	73·6 ± 7.2 (1)
Heparin plasma	0	0	0.21	79·1 ± 16·6
"	0	5	0.03	85.5 ± 7.9
"	0	10	< 0.01	77.9 ± 7.7
"	0	15	< 0.01	61·5 ± 5.2
"	0	20	< 0.01	54·7 ± 6.5
"	10	0	2	95.9 ± 12.5 (2)

Notes: (1) Ppt formed overnight

(2) Gave insoluble cryoprecipitate

2.2 Standard NY Process

The laboratory study involving the in-process addition of calcium or the replacement of citrate with a phosphate/sodium chloride solution is complete (see previous report and poster presented to ICTH meeting, July 1983).

The addition of calcium has been tested in production by splitting a production lot into 2 portions (ie NY 771 and 772). The first portion was processed normally (NY 771) while the second has $CaCl_2$ added with the Na citrate to maintain the concentration of ionised calcium (NY 772).

The results (below) confirmed the laboratory study with NY 772 showing an overall yield improvement of 20% according to the 1-stage clotting assay.

	NY 771	NY 772
1) Plasma volume (litres)	285	245
2) Volume dispensed (ml/l plasma)	40.90	44.41
3) FVIII potency (iu/ml) (20ml reconstitution from 35ml fill)		
1-stage (PFC)	15.20, 14.51	15.61, 17.27
1-stage (EBTS)	13.44, 11.34	16.52, 14·81
2-stage (EBTS)	12.60, 12.08	11.98, 11.34
VIIIR:Ag (EBTS)	37.31, 42.43	35.00, 36.7
4) Process yield (iu/l plasma)	372.2	446.6
5) FVIII content per vial (iu) - (PFC)	318	351

This improvement was not shown by the 2-stage clotting assay and this observation was confirmed by a stability study in which 2 vials from each lot were held for 24 hours at room temperature (results below).

Time (hrs)	VIII C iu/ml (%)		VIII C iu/ml (%)	
	1-stage assay (PFC		2-stage assay (PFC)	
	NY 771	NY 772	NY 771	NY 772
0, a sample	13.99 (100)	16.57 (100)	12.49 (100)	12.62 (100)
b sample	14.50 (100)	16.70 (100)	11.93 (100)	13.22 (100)
6, a sample	10.76 (76.9)	14.58 (88.0)	-	-
b sample	11.87 (81.9)	14.99 (89.8)	-	-
24, a sample	11.54 (82.5)	14.76 (89.1)	11.67 (93.4)	10.44 (82.7)
b sample	11.57 (79.8)	12.52 (75.0)	11.47 (96.1)	10.63 (80.4)

This assay discrepancy appears to be similar to that observed by Rock who has had to resort to a study of in vivo recovery to confirm her results (Thromb. Haem. 50, 109, 1983). It seems likely that the discrepanc which we have observed may also require an in vivo study to establish which assay is correct. 20 vials of each lot (NY 771/772) have been held for this purpose.

2.3/

2.3 Ca^{2+} During Pasteurisation

Addition of further calcium may be needed to protect FVIII:C in hot solutions (see 3.2 below).

3. New Process

3.1 Zinc Fractionation

The laboratory study of zinc fractionation is virtually complete (see poster presented at ICTH meeting). Scale-up studies have emphasised the importance of good mixing and careful reagent addition to avoid precipitation of FVIII.

3.2 Heat Treatment

Extensive studies have been carried out on the stability of FVIII:C and a range of model viruses to heating in solution in the presence of sorbitol and glycine.

Compared to an albumin control (caprylate stabilised) the sugar solutions (sorbitol or sucrose) showed substantial stabilisation of virus (vaccinia and mumps). Improved heating conditions have been identified to achieve further viral inactivation with little extra loss of FVIII:C (see table appended).

Even more severe heating results in substantial loss of FVIII activity and the improved conditions are probably the best that can be achieved without an unacceptable loss of yield.

These experiments were carried out in 50ml volumes. Scale-up to 500ml has given poor recovery of FVIII:C but results from further laboratory studies suggest that additional Ca^{2+} may be required to ensure proper stabilisation under these more severe heating conditions.

3.3 Finishing

3.3.1 Ultrafiltration

Scale-up studies of diafiltration for removal of zinc and sorbitol are complete. Problems of concentration polarisation were overcome by changing the type of pump used and by using a different diafiltration buffer.

One small batch of product prepared this way has been issued for clinical evaluation (heating : 60°C, 10 hrs).

3.3.2 Precipitation

Precipitation is being studied as an alternative to ultrafiltration Experiments have been carried out using methods similar to those of Behringwerke (US patent) and encouraging results have been achieved. This method could well be more attractive than ultrafiltration.

3.4 Neo-antigens (J. Dawes)

Immunoassays have been used to see if heating might have caused changes in the molecular structure of proteins in the product. No changes were observed after heating for 10 hrs at 60°C with:

FVIII:C, FVIIIR:Ag, fibrinogen, PF4, thrombospondin and β-thromboglobulin.

Samples heated under the improved conditions have shown no change in FVIIIR:Ag. The other proteins have still to be analysed.

4. Other Heating Methods

Other manufacturers are heating their products in the freeze dried state (Hyland, Armour). Experiments using this technique are being carried out using vaccinia and mumps to allow a comparison with heating in solution. Initial results suggest that the viral kill is less than that achieved by heating in sugar solutions at 60°C for 10 hrs.

SOLUTION	HEAT TREATMENT IN SOLUTION			
	60°C for 10 hours		Improved Conditions	
	Before Heating	After Heating	Before Heating	After Heating
Factor VIII (%, Mean & SD (n)) Prepared By Zinc Method				
Sorbitol/Glycine Stabilised	100	89.0±22.5(20)	100	76.9±7.2 (5)
Sucrose/Glycine Stabilised	100	67.5± 8.5 (4)	ND	ND
VIRUSES				
Vaccinia (pfu/ml)				
in FVIII (PFC Sorbitol/Glycine Solution)	$10^{8.5}$	10^{5}	$10^{7.5}$	$<10^{1}$
in Albumin (PFC Sorbitol/Glycine Solution)	$10^{8.5}$	$10^{4.5}$	ND	ND
in Albumin (Sucrose/Glycine Solution)	$10^{8.5}$	$10^{4.5}$	ND	ND
in Albumin (Standard Solution)	$10^{7.5}$	0	ND	ND
Mumps (pfu/ml)				
in FVIII (PFC sorbitol/Glycine Solution)	$10^{5.5}$	$10^{3.5}$	$10^{5.5}$	$10^{2.5}$
in Albumin (Standard Solution)	$10^{5.5}$	0	ND	ND
H. Simplex (pfu/ml)				
in FVIII (PFC Sorbitol/Glycine Solution)	$10^{6.5}$	$<10^{1}$	$10^{6.5}$	$<10^{1}$
in Albumin (Standard Solution)	$10^{6.5}$	0	ND	ND
Polio 2 (pfu/ml)				
in FVIII (PFC Sorbitol/Glycine Solution	10^{6}	$<10^{1}$	10^{6}	$<10^{1}$
in Albumin (Standard Solution)	10^{6}	0	ND	ND