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CONFIDENTIALSNBTS FACTOR VIII STUDY GROUPPROGRAMME OF PRIORITIES CONCERNING FACTOR VIII YIELDPLUS A BRIEF RESUME OF THE WIDER P.F.C. PROGRAMMEFACTOR VIII YIELD WORKING PARTY

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CONTENTS

1. INTRODUCTION
2. TOP PRIORITY PROJECTS
 - 2.1 Preventing Inactivation During Finishing
 - 2.2 Development of a High-Purity Method
 - 2.3 Conditions of Plasma Preparation and Storage
3. FUTURE STUDIES
4. THE WIDER PFC PROGRAMME
5. BIBLIOGRAPHY OF PFC INTERNAL R & D REPORTS (FVIII)

1. INTRODUCTION

Studies aimed at improving the yield and quality of Factor VIII concentrate produced at PFC are in progress. The current priorities in this work are detailed below. These essentially follow the points identified at the first meeting of the Study Group. (see minutes of meeting held on 28/1/82)

Some indication is also given of future studies that will be required if further yield improvement is needed.

A summary of all of PFC's FVIII work has been included so that the current projects can be appreciated in the context of what is essentially an on-going programme of study.

2. TOP PRIORITY PROJECTS

The main studies fit into three distinct project groups. These are:

- (i) Preventing inactivation during finishing (PFC)
- (ii) Development of a high-purity method (PFC)
- (iii) Defining conditions in plasma preparation and storage which influence the yield and quality of FVIII recovery in cryoprecipitate. (Edin RTC, PFC)

2.1 Preventing Inactivation During Finishing

We now know that the major remaining cause of yield loss within PFC is a citrate-mediated inactivation of FVIII:C.

Further studies have identified important benefits of citrate i.e.

- (i) Ease of filtration
- (ii) Prevention of clotting
- (iii) Increased solubility after drying.

We are now investigating the possibility of omitting citrate (either in whole or in part), substituting other reagents for citrate or counteracting the inactivating influence of citrate (eg by adding calcium to the process solution).

The reagents being studied include various combinations of:

- (i) Sodium chloride
- (ii) Sodium citrate
- (iii) Sodium phosphate
- (iv) Heparin
- (v) Glycine
- (vi) maltose
- (vii) Calcium chloride

Calcium addition has been carried out at three concentrations:-

- (i) Physiological
- (ii) 1/2 x physiological
- (iii)/

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- (iii) By back titrating ionised calcium levels to their original value.

Results are being assessed in terms of FVIII activity (1-stage assay and 2-stage assay) before and after freeze drying and in terms of final product solubility. (A number of other parameters are also being assessed eg Na, Cl, osmolality etc).

All of this is being done at a laboratory scale. When a preferred solution has been identified this should be tested at full-scale (1 batch initially). If this is successful then further batches may be tested. If calcium addition is used we may want to carry out a clinical trial to verify safety and in vivo recovery.

2.2 Development of a High-purity Method

The object of this work is to produce a FVIII concentrate with a low fibrinogen content but at a substantially higher yield than is achieved by the manufacturing methods currently in use. Ideally the method should also allow access to fibronectin.

A process based on metal-ion precipitation (zinc) of fibrinogen is presently being developed. A series of process stages can be identified:-

- | | | |
|-------|--|------------------------|
| (i) | Fibrinogen removal | 100% n.d. 10% in FVIII |
| (ii) | FIX (PTC) removal | 70% " 10% " |
| (iii) | Fibronectin removal | 100% " 10% " |
| (iv) | Reagent removal | |
| (v) | Finishing (ie stabilisation, concentration, filtration, freeze drying) | |
| (vi) | Hepatitis removal/inactivation | |

2.2.1 Fibrinogen Removal

The aim is to separate a significant proportion of the fibrinogen with little loss of FVIII. Behaviour is being studied at different concentrations of zinc but at neutral pH and room temperature (low temperatures are difficult to control accurately and take time to achieve at scale; neutral pH is recommended in the literature). Effects of mixing and kinetics are also being studied (all at very small scale, ie 30ml).

Some optimisation of the conditions selected will be needed on scale-up to both development (1 litre) and full scale (20 litres) processes.

FVIII analysis is being carried out by:-

- | | | | |
|------|-----------------|---|-------------------------|
| (i) | Clotting assays | - | 1-stage (PFC, EBTS) |
| | | | 2-stage (EBTS) |
| (ii) | Antigen assays | - | RAG, non-reduced (EBTS) |
| | | | RAG, reduced (HQ) |
| | | | CAG (HQ) |

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2.2.2 FIX Removal

Removal of PTC is necessary to avoid potential for activation, clotting and inactivation of FVIII:C. Problems here are related to process failure and to instability of the final product.

In the standard process, removal is achieved by $Al(OH)_3$ adsorption and this may be necessary in the zinc process. In this situation zinc fractionation would simply be an extension to our current process.

We have also noted that heparin (added to zinc supernatant at room temperature) throws down another precipitate. Early results show that FVIII is not precipitated but that FIX may be. Heparin may be interfering with FIX assays though, so further study is required. A FIX antigen assay would be useful. It is also possible that fibronectin is being precipitated; assay results are still awaited.

2.2.3 Reagent Removal

Experiments have shown that zinc remaining in the FVIII extract (about 50ppm) can be removed by diafiltration (90% removal against 7 volumes of buffer).

This will probably be the preferred technique as it avoids the addition and removal of further reagents (eg ion exchange) and is compatible with a subsequent concentration stage (ultrafiltration).

It is not clear what level of zinc could or should be tolerated in the product. A toxicity review is in progress but local medical opinion would also be of interest.

2.2.4 Finishing

Studies here will depend largely on the results of 2.1 above.

2.2.5 Hepatitis

Zinc has been reported to be a potent precipitating agent of virus (Cohn, 1954).

A low concentration of fibrinogen may also make pasteurisation more feasible. Hence the process presents two possibilities for solving the hepatitis problem, both of which should be non-specific and could therefore apply to all the different forms of viral hepatitis (see safety action working party).

2.2.6 Further Work

The accuracy of clotting assays in the presence of zinc may be doubtful. Study of this may be required, this may have to be backed up by studies of in vivo recovery.

2.3 Conditions of Plasma Preparation and Storage

There may be some overlap of interest with the "plasma preparation" group. However, the "yield" group are particularly concerned in assessing cryoprecipitate recovery from high quality plasma (eg plasma destined for thaw-siphon cryo) under defined optimal thaw conditions (eg thaw-siphon).

Initial/

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Initial experiments will be concerned with the effect of temperature history on the recovery and composition of cryoprecipitate with a view to defining optimal conditions of freezing temperature, freezing rate, storage temperature and time.

To avoid interference from other parameters initial studies will use pooled fresh non-frozen plasma with appropriate experimental controls.

Further studies will cover the effect of plasma additives (eg heparin).

3. FUTURE STUDIES

Current PFC process yield is 45% of the FVIII monitored in the feedstock entering process. It is estimated that a solution to the problem 2.1 above could increase this figure to about 60%. Further improvement will require a selective redistribution of FVIII from the cryosupernatant into the cryoprecipitate. This may be achieved in part by the studies 2.3 above but a better understanding of the mechanism of cryoprecipitation is likely to be required.

This is considered to be a phenomenon of salting-out due to the concentration of salts during freezing/thawing. Study of this mechanism may be needed to identify reagents or conditions which will specifically promote the precipitation of FVIII and, at the same time, protect the molecule.

4. THE WIDER PFC PROGRAMME4.1 Introduction

This section simply lists the various studies undertaken at PFC in recent years. Present day priorities are noted, but these do tend to change frequently according to either our own data or to information provided by others. In a number of these projects we have enjoyed the assistance of scientific colleagues from both within and outwith the SNBTS.

4.2 History

Early FVIII concentrates at PFC (then the Blood Products Unit at Edinburgh BTS) were prepared from Cohn fraction I using aseptic pooling techniques (Cumming et al. Vox Sang. 10, 687-699, 1965). This product suffered from problems of low potency and poor quality assurance as it could not be filtered for bacterial removal. It was therefore replaced in 1974 with the intermediate purity concentrate. (Newman et al. B.J. Haem. 21, 1-20, 1971). Studies at this time on the high purity method of Newman et al gave very poor yields and this method was not pursued any further.

Following transfer to the new Centre at Liberton in 1975 a programme of study was initiated to investigate and improve the standard intermediate-purity process. Some occasional work has continued on alternative methods but this has had a low priority until very recently as the ever increasing demand for FVIII (estimates in IU per 10^6 pop/yr were 0.80 in 1976, 2.75 in 1981 projected to 3.75 for 1991) has meant that emphasis has been placed almost entirely on yield rather than quality.

4.3 Plasma Studies4.3.1 Factor VIII Content

Initiated 1975 to study reasons for low levels of FVIII:C

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(i.e. 0.6 u/ml) with the aim of achieving high quality plasma for fractionation.

Progress: Study considered complete in 1979 with routine 6 hr CPD plasma giving 0.90 u/ml prior to freezing at RTC's and 0.84 u/ml on entry to process at PFC. Needs to be reconsidered following dramatic fall in FVIII:C levels in 1981. Positive protection of FVIII:C may be required. Priority: Very high. See plasma group

4.3.2 Plasma Freezing

Theoretical engineering study carried out in 1974 to identify key factors influencing rate of freezing. Evaluation of purpose built equipment 1981.

Progress: Engineering principles well defined and can be applied as needed.

4.3.2 Length of Frozen Storage

Study initiated 1980 to evaluate yield and quality of cryo-precipitate from plasma collected by plasmapheresis.

Progress: Preliminary experimental studies confirm the view that the fibrinogen content of cryoprecipitate varies according to conditions of plasma storage. Not necessarily a problem if high purity method (2.2) removes fibrinogen. Must be considered in conjunction with 2.3 above. Priority: Medium.

4.3.4 Pre-process conditioning

Initiated in 1979 to study effect of pre-process temperature on FVIII yield and quality of cryoprecipitate.

Progress: Optimal conditions defined but mechanisms not understood. Further study needed. Priority: Low

4.3.5 Pack Removal

Initiated 1978 to establish a system for the rapid cleaning and stripping of packs without detriment to FVIII yield or quality.

Progress: Various methods studied (eg liquid N₂ fracture, ethanol washing) but results not satisfactory. Recent work on pack tearing and U.V. sterilisation encouraging. Priority: Very high

4.4 Evaluation and Improvement of the Standard Process

4.4.1 Plasma Thawing

Initiated 1976 to design and study continuous thawing as a means of achieving improved quality and yield.

Progress: Preliminary engineering design completed 1977. Experimental engineering research completed 1978. Pilot-scale production 1979. Full-scale production 1981.

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4.4.2 Cryoprecipitate Washing

Initiated 1979 as a potential means of improving quality.

Progress: Satisfactory wash solutions determined and some equipment developed. Suspended because of FVIII:C inactivation. Further study could now resolve this problem but the method may not be necessary if a high purity method is developed.

Priority: Low

4.4.3 Cryoprecipitate Extraction

Initiated 1979 to re-determine optimal conditions for continuous thaw cryoprecipitate.

Progress: Completed 1980

4.4.4 Aluminium Hydroxide Adsorption

Initiated in 1979 to re-determine optimal conditions for continuous thaw cryoprecipitate.

Progress: Completed 1979. Further studies concerning influence of plasma quality are required. Priority: Medium

4.4.5 Aluminium Contamination

Initiated 1978 to establish the mechanism by which aluminium reaches the final product.

Progress: Substantial work carried out but solution to the problem not yet achieved. Suspended 1980 when PFC levels were considered to be within the limits acceptable to USA BoB. Further work may be required. Priority: Low

4.4.6 Inactivation

Initiated 1981 to determine mechanism of loss during finishing.

Progress: Mechanism identified. Study to prevent inactivation in progress see 2.1 above. Priority: Very high

4.4.7 Concentration

Initiated 1981 to investigate the use of ultrafiltration for concentrating intermediate-purity solutions.

Progress: Preliminary laboratory study completed. Further progress requires solution to 4.4.6 above. Priority: Medium

4.4.8 Freezing

Initiated 1980 to study effect of freezing rate on solubility of freeze dried powder.

Progress: Very high solubility if frozen in liquid nitrogen, but thin skin of poorly soluble material at surface. Further progress/

7.

progress probably requires a high purity concentrate.

Priority: Low

4.4.9 Freeze Drying

Initiated 1982 because of problems of quality and capacity.

Progress: Immediate problems resolved but optimal conditions still to be determined. Will require R & D freeze drier (available 1983). Priority: Medium

4.5 Other Fractionation Methods

4.5.1 Cryoprecipitate Enhancement

Initiated 1976 to study effect of heparin and PEG additives to plasma.

Progress: Discontinued because of poor yield in final product. May need to be reconsidered because of recent widespread interest.

Priority: See 2.3 and 3 above

4.5.2 Polyelectrolyte Adsorption

Initiated 1974 to study solid-phase adsorption, in conjunction with New York University.

Progress: Discontinued because of poor yield and uncertain supply of reagent. Method now being pursued by Speywood and may still be of interest if good results achieved.

4.5.3 Cold/Acid Extraction

Initiated 1976 to study factors influencing the separation of FVIII and fibrinogen.

Progress: Considerable laboratory data but difficult to reproduce on scale-up. Similar studies published by others (Smith et al. Transfusion 19, 299-306, 1979). Priority: Suspended as 2.2 is more attractive.

4.5.4 Metal-Ion Precipitation

Initiated 1981. See 2.2 above.

4.6 Other Studies

4.6.1 Pyrogen Removal

Initiated 1980 to study the use of filter media for pyrogen removal.

Progress: Positive results with model solutions but not yet substantiated with true solutions. Priority: Medium

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4.6.2 Ionising Radiation

Initiated 1980 as a potential means of inactivating hepatitis virus.

Progress: See safety action working party reports.

4.6.3 Pasteurisation

Initiated 1981 as a potential means of inactivating hepatitis virus.

Progress: Published method requires high purity concentrate.
See safety action working party reports.

5. BIBLIOGRAPHY OF PFC INTERNAL R & D REPORTS (FVIII)

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