TED TUDDENHAM - TORONTO LECTURE
MONO - VIII:C

It was at the 1976 World Federation of Haemophilia Conference in Kyoto that Dr. Alan Johnson of N.Y. University first outlined a new fractionation process using solid-phase ion exchange resins known as polyelectrolytes.

After almost 5 years of further intensive research and scale up we can now report that polyelectrolytes do indeed offer a viable alternative to conventional processes, giving extremely pure fractions at high yield.

We have concentrated on the production of Factor VIII:C of both human and animal origin. As you will see the products are unique, simple to produce and efficacious.

Before giving you the process details I would like to show you the basic structure of the polyelectrolytes.

SLIDE 1

X is the backbone basic polymer (Ethylene Maleic Anhydride).
Y is the DIMETHYLAMINOPROPYLIMIDE which is positively charged.

E5, the polymer used for Factor VIII preparation, has 5% of the anhydride groups substituted with the DMAPI groups - to give a low positive charge.

The unreacted anhydride groups are blocked to prevent hydrolysis Methoxy propylamine (MDPA).

SLIDE 2

- This slide illustrates in simple terms how a protein is adsorbed to the polyelectrolyte and eluted.

- In the case of Factor VIII, at pH 6.8 and 0.3M NaCl ionic strength, the Factor VIII:C component of the Factor VIII aggregate is selectively adsorbed to the resin.

SLIDE 3

Shows the outline process.
We used cryoprecipitate as the starting material, since this is currently the conventional source of almost all Factor VIII products.

- The process is identical for both pig and human up to the PEG precipitation.

- Factors II, VII, IX and X are removed with Aluminium Hydroxide.

- Solution adjusted to pH 6.8 and 0.3M NaCl.

- The FVIII:C is adsorbed to the polyelectrolyte.

- Supernatant contains, FVIIIR:Ag, vW activity and in the case of pig PAF activity. It is also rich in fibronectin

- Washing removes further extraneous proteins.

- 1M NaCl at pH 6.5 elutes the FVIII:C.
  (Notice the change in molarity 0.3 to 1.0)

- 20% PEG precipitates the FVIII:C.

- Porcine is further processed through Sepharose 4B, without significant loss in yield, to reduce the porcine protein content even further.

The operation has significant advantages over current fractionation methodology.

1) Higher yields - 35% from cryo
   50% from plasma.

2) Ultra high purity fractions.

3) Simple process, less labour, energy and capital required.

4) Operates at room temperature.

5) Can be performed at small scale, 1 Kilo cryo or 1 litre plasma.

It is thus an entirely feasible process for individual blood centres or small Governmental fractionation units.

SLIDE 4

Shows a typical analysis of human and porcine FVIII:C products.

- Units are presented at 25 per ml to conform to current practice.

- Specific activity of human is 10 u/mgm.

- Specific activity of pig, due to S4B is 150 u/mgm.

- PAF is virtually eliminated from the porcine.

- RAG is virtually eliminated from the human.

- Isoagglutinins are absent.

- The product is extremely soluble, unless it has been stored incorrectly.

Both products have now been used clinically and some of the results are being presented here and in Toronto. Half-life is of the same order as present FVIII products, ranging between 8 and 16 hours, depending on individual patient response.

Porcine FVIII is of course used for the treatment of inhibitor patients, thrombocytopaenia has been virtually eliminated as a side-effect and other adverse reactions are much less severe than with previous animal preparations. Haemophilia centres which have used Hyate:C report dramatic improvements in the life-style and morale of their inhibitor patients. The possibility of porcine FVIII:C being used for non-inhibitor patients in countries with a shortfall of human FVIII should now be seriously considered. Of course, viral hepatitis is not present in porcine plasma and the product thus presents no risk of infection.

In vitro tests have demonstrated that Hepatitis B surface particles do not bind to the E5 polyelectrolyte under the conditions used for the production of FVIII:C.

In trials, using heavily infected cryo, simple washing of the polymer reduced Hepatitis B below detectable levels, leaving the Factor VIII:C intact. There is the encouraging possibility of concentrates with a reduced risk of hepatitis transmittal. It has also been hypothesized that the immunogenic character of PE FVIII:C may differ from that of the conventional FVIII agglomerate. Antibody response may be changed.

Only further clinical experience will prove or disprove the latter points but despite this we feel that a higher purity Factor VIII concentrate at a yield significantly above the conventional, offers excellent prospects for the treatment of haemophilia in the 1980s.