0030

10:00 h

BINDING TO PHOSPHOLIPID PROTECTS FACTOR VIII FROM INHIBITION BY HUMAN ANTIBODIES. T.W. Barrowcliffe, E. Gray and G. Kemball-Cook. Division of Blood Products, National Institute for Biological Standards and Control, London NW3 6RB, U.K.

Previous studies with activated Factor IX concentrates have suggested that they may contain a form of Factor VIII clotting activity (VIII:C) which is partly protected from inactivation by antibodies. A possible mechanism for such protection is binding to phospholipid. The interaction between Factor VIII, phospholipid and human antibodies to Factor VIII was studied by a two-stage clotting assay, and by a fluid-phase immunoradiometric assay for Factor VIII clotting antigen (VIII C:Ag).

In the two-stage thrombin generation assay, Factor VIII:C was rapidly destroyed by human antibodies, even in the presence of optimal phospholipid. However, pre-incubation of Factor VIII with phospholipid before addition of antibody protected the Factor VIII from inactivation, resulting in the production of much more thrombin.

In assays of VIII C:Ag, pre-incubation of Factor VIII with phospholipid before addition of labelled antibody reduced the amount of detectable antigen. The reduction was greater with increasing phospholipid concentration, up to 60% of the original antigen being 'lost' at a total phospholipid concentration of around 250 µg/i.u.

These results suggest that human antibodies to Factor VIII are directed largely at its phospholipid binding site. The protection of Factor VIII from inactivation by complexing with phospholipid could have important clinical applications in treatment of haemophiliacs with inhibitors.

0031

10:15 h

POLYVINYLPYRROLIDONE (PVP).A NEW PLASMA FRACTIONATION AGENT. G.Casillas,C.Simonetti. Departmento de Bioquímica. Instituto de Investigaciones Hematológicas.Academia Nac. de Medicina.Buenos Aires.Argentina.

The techniques used currently to prepare F.VIII concentrates are chiefly based on alcoholic,cryo or PEG precipitation,

Here we described the preparation of F.VIII concentrate from human and bovine plasma using a new precipitating agent (PVP). The precipitation curves of F.VIII and fibrinogen in function of the PVP concentration,pH and temperature were studied and then the optimum conditions as regard recovery,purification and fibrinogen content were adjusted.

A concentrate (1/10 de volume of the original plasma) containing 8U F.VIII/ml (recovery 80%), 16mg/ml protein and 7mg/ml fibrinogen (recovery 20%) is obtained from human plasma in the following conditions: 4% PVP concentration, 5°C and extraction of the precipitate at 2-3°C with a 0.15M NaCl , 0,5M glycine solution.

For bovine plasma the conditions are similarexcept for the precipitation,which is performed at room temperature (15-20°C).The recovery of F.VIII and fibrinogen is 90% and 10% respectively and the protein content 12mg/ml.Small variations in the pH of plasmas do not modify the results.

0032

10:30 h

RESPONSE TO INFUSION OF POLYELECTROLYTE FRACTIONATED HUMAN FACTOR VIII CONCENTRATE IN HUMAN HAEMOPHILIA A. P.B.A. Kernoff, R.S. Lane, S. Middleton and E.G.D. Tuddenham. Haemophilia Centre & Haemostasis Unit, Royal Free Hospital, London, and Blood Products Laboratory, Elstree, Herts., England.

Plasma fractionation using polyelectrolytes (PEs) has potential advantages which include simplicity of procedure, high product purity, increased yield and removal of hepatitis viruses. The purpose of this study was to assess the in-vivo response to PE-fractionated human factor VIII concentrate (PE VIII) given to 3 volunteers with severe haemophilia A. PE VIII fractionated from bulk cryoprecipitate had a high specific activity (6.74 u/mg protein), no detectable isoagglutinins, a low fibrinogen content, high ratio of VIII coagulant activity (VIII:C)to VIII-related antigen (VIIIR:Ag), and a ratio of VIII:C to VIII coagulant antigen (VIII:CAg) of approximate unity. Single infusions, each of about 2000 i.u.(25 - 40 i.u./kg), were administered i.v. over 10 mins. There were no clinical, haematological or biochemical adverse effects during the 48 hr. post-infusion observation period. Immediate post-infusion recoveries of VIII:C were 119, 102 and 70 per cent with late phase half disappearance times of 17, 19 and 17 hours respectively. These values were similar to those obtained after infusion of intermediate-purity factor VIII concentrate to the same patients, and are in accord with the results of previous studies using conventional concentrates. PE VIII has potential as a therapeutic material for patients with haemophilia A.

0033

10:45 h

A NEW CLINICAL PREPARATION OF HIGH-PURITY PORCINE FACTOR VIII:C CONCENTRATE. W.P. Costello, S.M. Middleton & R.G. Malia+. Speywood Laboratories, Chancel House, East Street, Bingham, Notts and +Haematology Department, Hallamshire Hospital, Sheffield, England.

A polyelectrolyte-fractionated porcine Factor VIII concentrate (Hyate:C, Speywood Laboratories, average specific activity: 10 U Factor VIII:C/mg, platelet aggregating factor (PAF): 0.25 U/ml) has been recently available. Clinical use of this product for the treatment of patients with antibody to Factor VIII (13 haemophilic and 1 non-haemophilic patients for a total of 26 courses of treatment) has shown that: 1/ multiple courses of therapy can be given with both good clinical tolerance and good clinical efficacy, 2/ with one exception, no patient developed thrombocytopaenia and 3/ with the exception of one case, in which the patient has been treated with human Factor VIII prior to the infusion of porcine Factor VIII. the expected anamnestic response did not occur. These clinical findings prompted us to further purify the porcine Factor VIII concentrate for clinical use.

Cryoprecipitate, prepared from frozen porcine plasma, is dissolved in low ionic strength Tris buffer at pH 6.8 and adsorbed with aluminium hydroxide gel. The ionic strengthis then adjusted to 0.3 M NaCl before loading on a column of charged and equilibrated polyelectrolyte E-5. After washing the column with 4 bed volumes of buffer, the proteins were eluted with 1 M NaCl at pH 6.5 and precipitated by 20 % polyethylene glycol 4000. The redissolved precipitate is then chromatographed on a preparative column of Sepharose CL-4B. The void volume is sterile-filtered and lyophilised. The new preparation has a specific activity of approximately 100 U Factor VIII:C/mg with a PAF level of 3.3 U/ml. This represents an improvement of 10 fold in specific activity over the existing concentrate.

It is expected that the new concentrate will also show low immunogenicity which can only be revealed by clinical results. A clinical trial is envisaged within the next two months.