Preparation of Factor VII concentrate by immunoaffinity chromatography

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ABSTRACT
In this research, immunoadfinity chromatography was used to purify factor VII from prothrombin complex (PPSB), which contains coagulation factors II, VII, IX and X. For this purpose DEAE-Sephadex and CNBr-activated Sepharose 4B gels were used. Specific activity of factor VII concentrate increased from 0.16 to 55.6 with purification-fold of 347.5 and the amount of activated factor VII (FVIIa) was found higher than PPSB (4.4-fold). Results of electrophoresis on agarose gel indicated higher purity of FVII compared to PPSB and these finding revealed that factor VII migrated as alpha-2 proteins. In order to improve viral safety, solvent-detergent treatment was applied prior to further purification and elimination of tween 80 was nearly complete (2 µg/ml). Factor VII concentrate is used for patients with factor VII deficiency and also for hemophilia patients with inhibitors.

Introduction
Human coagulation factor VII (FVII) is a glycoprotein with a molecular mass of 50 kDa, is synthesized in the liver and it circulates in the blood at a plasma concentration of 0.5µg/ml. In 1972 Dike, Bidwell and Rizza reported the preparation and clinical use of a concentrate of factor VII as a by-product of the preparation of a therapeutic concentrate of factors II, IX and X by adsorption on DEAE-Cellulose. In 1973, it was concluded that DEAE-Sephadex was more suitable than DEAE-Cellulose for routine large-scale production of the prothrombin complex. In 1980, batch adsorption on DEAE-Sepharose CL-6B followed by elution on a chromatographic column, concentrated factor VII about 25-fold without a need for further dialysis or concentration steps. In 2003, an activated Factor VII (FVIIa) concentrate, prepared from human plasma on a large scale, became available for clinical use for haemophiliacs with antibodies against FVIII and FIX. The management of bleeding episodes in patients with inhibitors may require different therapeutic approaches, among which factor VIIa-6-10 and prothrombin complex concentrates 11-12 have been successfully used. FEIBA (Factor Eight Inhibitor Bypassing Activity, Immuno, Vienna, Austria) is an activated prothrombin complex concentrate which has been widely used in the treatment of hemophilia patients with inhibitors. Factor VII concentrates are used in patients with congenital or acquired factor VII deficiency or treatment of hemophilia patients with inhibitors 4-6. It was shown that high levels of FVIIa in prothrombin complex concentrates containing factor VII, may contribute to the thrombogenic potential of these preparations, therefore purifying FVII from PPSB should improve its thrombogenicity. This study was planned so as to improve tolerance and safety in the treatment of patients with preparing highly purified factor VII from PPSB by using immunoadfinity chromatography technique.

Material and Methods
Prothrombin complex preparation:
PPSB was prepared from plasma conventionally by DEAE-Sephadex method (Na citrate 0.01 M, pH:7.0, NaCl 2M) and was used as the starting material for purification of coagulation factor VII. Prothrombin complex was treated with a mixture of 0.3% tri-(n-butyl) phosphate (TNBP) and 1% Tween 80 at 24°C for 6 h with constant stirring.

Preparation of specific antibody against human factor VII
Antiserum against human FVII (Assera factor VII, Stago) was further purified by ammonium sulphate 50%, after centrifugation dialysed in coupling buffer (0.1 M NaHCO3, pH 8.3 containing 0.5 M NaCl).

Preparation and packing the gel
Preparation of the gel (2 g powder), coupling the ligand (Assera factor VII) with coupling buffer for one night, blocking excess remaining groups with Tris buffer (pH:8, 0.1 M) for 2 h and packing of the gel were performed according to the brochure of the kit.

Immunoadfinity chromatography
The mixture of PPSB (50 ml) in the PBS buffer (pH: 7.4) was filtered (0.22 µ) and then chromatographed (Pharmacia LKB Fraction Collector 2210) on a column (K 9/15 Pharmacia) containing CNBr-activated Sepharose 4B coupled with specific antibody. Flow rate was adjusted to 0.75 ml/min. After washing step, elution was performed by glycine buffer (0.1 M, pH:2.5) and FVII collected in collection phosphate buffer (1.0 M, pH:8) fractions.

Clotting assays
Factors II, IX, VII, X and VIIa were assayed on the fractions by one stage clotting assay method using Stago kits. Fractions of 48-50 were pooled and lyophilized as a factor VII concentrate.

Agarose gel electrophoresis
This method was performed using barbital buffer (pH:8.6) at 220 V for 35 min with Ciba Corning equipment.

Determination of Tween 80
TWEEN 80 was measured spectrophotometrically at 535 nm.

Results and Discussion
Ellution pattern of FVII from PPSB is shown in Fig.1. In this pattern one major peak of FVII (Fractions 48-50) and the minor peak of FIX (Fractions 46-51) has been shown, because the activity of other coagulation factors was lower. Other unadsorbed or unwanted proteins have been removed in breakthrough and other fractions.
Our study demonstrated that factor VII concentrate, essentially free of factors II, IX and X, can be further purified from prothrombin complex by immunoaffinity chromatography, and a virus inactivation step of solvent-detergent treatment could also be included.

References: