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IN VITRO COMPARISON OF ACTIVATED & NONACTIVATED PROTHROMBIN COMPLEX CONCENTRATES (PCC). J. Lazerson, C.F. Abildgaard, J. Harrison. Department of Pediatrics, University of California-Davis, Sacramento, CA 95817

In order to determine whether any *in vitro* test is related to clinical efficacy of PCC we compared non-activated PCC (Konyne-Cutter, Proplex-Hyland; Prothromplex-Immuno) with activated PCC (Autoplex-Hyland, FEIBA-Immuno, AntiInhibitor Correctional Concentrate (AICC)-Cutter). The non-activated partial thromboplastin time (NAPTT), factor VIII correctional activity (FVIIICA) by the Hyland method, and factor VIII inhibitor bypassing activity (FEIBA) by the Immuno method were performed on all PCC according to the manufacturer's specifications, utilizing their reagents. The results are reported as averages as interlot differences were minimal.

PCC no. lots tested	NAPTT (sec) 1/10 (dilut)	1/100	FVIIICA u/ml	FEIBA u/ml
Konyne (4)	189	218	7.7	non-linear
Proplex (2)	339	239	7.7	
Prothromplex (2)	293	237	9.0	
Autoplex (2)	34	47	20.1	34
AICC (2)	32	123	19.8	25
FEIBA (2)	53	165	15.3	16

No differences in results were obtained in FVIIICA assay of Autoplex whether inhibitor plasma or noninhibitor (factor VIII deficient) plasma was used as substrate, in contrast to the FEIBA assay where substitution of non-inhibitor plasma resulted in a threefold decrease in final units of FEIBA (i.e. 5 units vs 16 units).

It is concluded that the non-activated PCC contain 1/3 to 1/2 the factor VIII correctional activity of the activated PCC and that the NAPTT depending on the dilution selected is only a poor correlate of either. The relationship of the assays to clinical efficacy remains unknown.

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Investigation of the binding of calcium to prothrombin by two dimensional immuno electrophoresis.

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The reported binding constant of calcium to purified prothrombin based on equilibrium dialysis is $K<10^4$. This study investigated the ability of EDTA, EGTA, to remove calcium from the normal prothrombin, in the plasma of patients treated with warfarin.

Coumarin plasma was obtained from freshly drawn venous blood of patients on warfarin. This plasma contains prothrombin with no GLA's, which does not bind calcium, and prothrombin with GLA's, which does bind calcium. The plasma anti coagulated with EDTA or EGTA was subjected to two dimensional immuno electrophoresis (2DIE). In parallel experiments the plasma was dialysed against normal saline containing EDTA 2mM for 24 hours. The dialysis was performed at pH 8,9 and 10, and the dialysed plasmas, subjected to 2DIE.

RESULTS All plasmas, irrespective of the anti coagulant or pH of dialysis exhibited the same two prothrombin peaks on 2DIE, which indicates that the normal prothrombin had retained its bound calcium. This suggests that native prothrombin binds calcium with a K assos$>10^{10}$ the binding constant for EDTA and calcium at pH10.0.

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PURIFICATION OF 1- AND 0-GLA PROTHROMBINS AND THEIR DIFFERENTIATION WITH NORMAL AND OTHER DICOUMAROL-INDUCED PROTHROMBINS. O.P.Malhotra. Medical Research Service, Veterans Administration Medical Center and Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106 U.S.A.

In normal prothrombin, 10-glutamyl residues present in the amino portion of the molecule are carboxylated to form γ-carboxyglutamic acids (gla). Dicoumarol, an antagonist of vitamin K, induces the production of (partially) acarboxylated atypical prothrombins. In addition to our atypical varieties, viz. 7-,5- and 2-gla prothrombins, we have isolated two more atypical molecules, one containing 1.0±0.1 gla (1-gla prothrombin) and the other approximately 0.25 gla (0-gla prothrombin). These two variants adsorbed onto alumina Cγ-gel (Bio·Rad), similar to 2-gla protein, but were derived from 40 to 50% $(NH_4)_2SO_4$ saturation. The purified materials, obtained after isoelectric precipitation followed by preparatory polyacrylamide-gel electrophoresis and heparin agarose chromatography, showed a single component by analytical disc-gel electrophoresis both in the presence or absence of sodium dodecyl sulfate (SDS) and contained antigenic activity comparable to that of normal prothrombins.

The pI's of the two variants by column electrofocusing were each 4.835±0.015. Similarly, the two proteins did not reveal any difference in electrophoretic mobility; however, their prothrombin fragments 1 (F_1, residues 1-156) did — 0-gla F_1 moved slower than 1-gla F_1. Employing anti-(normal) prothrombin sera with Ca^{2+}, the two, 0- and 1-gla, F_1's produced vaguely visible immunoprecipitates which were definitely lighter than all the other F_1's including 2-gla. These results confirm that not only are multiple forms of atypical prothrombin induced by dicoumarol but also that gla does affect the immunochemical properties of the gla-containing fragment.

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EFFECT OF A NON-ACTIVATED PROTHROMBIN COMPLEX CONCENTRATE (PROTHROMBINEX) ON PLATELETS: IN VITRO AND IN VIVO STUDIES. H. Ekert, F.L. Dean and J.L. Lane. Department of Clinical Haematology and Oncology, Royal Children's Hospital, Melbourne, Victoria, Australia.

Chromatography on Sephadex G-200 of the prothrombin complex concentrate, prothrombinex (Px) showed it to have inhibitor and potentiator fractions when tested by the non-activated partial thromboplastin time (NAPTT). The inhibitory effect was related to the heparin content of Px, as it was removed on ECTEOLA-cellulose. The potentiator fraction clotted fibrinogen and this could be inhibited by hirudin. Thrombin-like activity of this fraction was shown using chromogenic substrates. The effect of the potentiator fraction in the NAPTT was markedly enhanced by platelets. Diluted Px and the potentiator fractions caused aggregation of washed platelets which could be inhibited by hirudin. Aggregation was independent of the prostaglandin pathway, as it occurred in washed aspirin-treated platelets. Neither diluted Px nor potentiator fractions aggregated platelets in platelet rich plasma. Infusion of Px was followed by a rise in β-thromboglobulin (β-TG) in 2 of 3 patients two minutes after infusion. This rise could not be ascribed to the presence of β-TG in Px or to the heparin present in Px. These findings suggest that Px has a thrombin-like activity and that its mode of action in patients with factor VIII antibodies may result from its effect on platelets and their interaction with coagulation enzymes.

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