

CLEAVAGE OF HUMAN PROTHROMBIN BY PARTIALLY PURIFIED ECHIS CARINATUS VENOM. E. Briët, M.J. Griffith, P.B. Soule, S.H. Jordan, K.M. Braunstein and H.R. Roberts. Center for Thrombosis and Hemostasis. University of North Carolina, Chapel Hill, N.C., USA.

A number of studies have been reported in which the pro-coagulant activity of *Echis carinatus* venom (ECV) has been used to investigate the structure-function of human and bovine prothrombin (II). While it is clear that ECV activates II by cleaving between the A chain-B-chain of the thrombin region of the zymogen, the literature is not clear on the activity of ECV in terms of cleaving at other sites. Specifically, one report suggested that ECV cleaves II between the Fragment 1 and prethrombin 1 regions (F 1-Pre 1) of the zymogen and that this cleavage is required for further cleavage and activation of II. Another report suggested that ECV does not cleave the F 1-Pre 1 bond at all. The ambiguity has arisen because of the well-known sensitivity of the F1-Pre 1 bond to cleavage by thrombin and the inability of most thrombin inhibitors to block thrombin activity during the course of II activation by ECV. To this point, diisopropylphosphorofluoridate, isoleucyl-propyl-arginine chloromethyl ketone, and benzamidine did not prevent significant F 1-Pre 1 cleavage by ECV in the present study. Dansyl-arginine-N-(3-ethyl-1, 5-pentanediy) amide (DAPA) however, appeared to afford nearly complete protection against F 1-Pre 1 cleavage during ECV activation of II. Since some F 1-Pre 1 cleavage was observed even in the presence of $10^{-3}M$ DAPA ($K_i = 10^{-7}M$) it appeared that ECV might cleave this bond at a relatively slow rate. This was confirmed by the results of experiments in which the rate of F 1-Pre 1 cleavage in the presence of DAPA was found to be proportional to the concentration of ECV. In addition, EDTA, which inhibits ECV activity, blocked further F 1-Pre 1 cleavage at a time when the conversion of prothrombin to meizothrombin was complete. Human prothrombin appeared to be much more susceptible to F 1-Pre 1 cleavage by ECV than bovine prothrombin. Our results suggest that the activation of prothrombin by ECV does not require F 1-Pre 1 cleavage by ECV as suggested by other investigators.

AN ACQUIRED INHIBITOR TO COAGULATION FACTOR II. M.F. Scully V. Ellis, V.V. Kakkar, G.F. Savidge¹, Y.F. Williams², H. Sterndale². Thrombosis Research Unit, King's College Hospital Medical School, London, England. ¹Haemophilia Centre, St. Thomas' Hospital, London, England. ²Margate Hospital, Margate, England.

A 77 year old woman with symptoms of GI tract bleeding, haematuria, bruising and profuse bleeding after dental extraction was found to have a prolonged thrombin time (>90 secs) but normal reptilase time and fibrinogen level. Both prothrombin time and KCCT were prolonged to greater than twice normal values. She had no previous history of bleeding problems and no drug history apart from analgesics.

On investigation of citrated plasma samples an antithrombin III level as measured in a chromogenic heparin cofactor assay of 607% was found but measured immunologically of 120%. Upon chromatography on Ultragel ACA44 two peaks of antithrombin activity were observed one eluting in the position of normal antithrombin III immunologically. The activity of this fraction differed according to whether the antithrombin assay was a progressive type or heparin cofactor. The other much larger peak eluted in this second peak off ACA44 and the activity was not susceptible to heparin. The activity in this peak was pooled and chromatographed on DEAE Sephacel. It eluted in 0.0175 M Na (P) pH 6.8 as a single peak coincident with antithrombin activity. On SDS electrophoresis a single band was obtained. This fraction corresponds to IgG. Less than 10% of the activity was eluted with 0.08 M (P) pH 6.6 in the fraction containing IgG and IgA.

The purified IgG directly inhibited thrombin. When added to normal plasma and prothrombin measured using activated factor Xa, complete loss of prothrombin was observed at dilutions of the inhibitor corresponding to 25% of that found in the patient plasma. The inhibitor had no effect on the rate of generation of factor Xa in normal plasma (S2222) as initiated by thromboplastin nor on the activity of purified factor Xa. The evidence suggests that this is the first report of an acquired inhibitor specific for factor II.