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ACQUIRED FACTOR X DEFICIENCY IN MULTIPLE MYELOMA: A COMPLETE RESPONSE TO CHEMOTHERAPY. M. Kos, K. Geißler, K. Ratheiser, I. Pabinger, Ch. Korninger, K. Lechner. 1st Dpt. of Medicine, University of Vienna, Austria.

A 64 year old women without any previous history of bleeding diathesis presented with bone pain and gastrointestinal bleeding. An isolated severe factor X deficiency (factor X activity 0.5%, factor X antigen less than 12.5%) was found. No inhibitor that inactivated factor X in vitro or interfered with factor X assay could be demonstrated. Substitution therapy with a prothrombin complex preparation containing factor X (PPSB Biotest) was given. Factor X recovery in the first 2 days was lower than expected (below 20%) and half life of factor X was shortened (150 minutes). Subsequently, a diagnosis of multiple myeloma (light chain myeloma, type kappa) was made. Amyloidosis was excluded by electronmicroscopic examination of rectum biopsies. Chemotherapy according to the M2 protocol (Case et al) was initiated. Factor X recovery improved dramatically within 2 weeks and there was a continuous increase of factor X activity and antigen during chemotherapy. After 6 courses a complete haematological remission (less than 5% plasma cells in the bone marrow, disappearance of light chains) was obtained and factor X activity and antigen returned to normal.

Isolated factor X deficiency is a wellknown complication of amyloidosis. To our knowledge, this is the first case of factor X deficiency in multiple myeloma without amyloidosis. The complete normalization of factor X after successful chemotherapy indicates that plasma cell proliferation may have been the cause of the factor X deficiency. Binding of factor X to plasma cells containing light chains could be a possible explanation, and we are currently examining this hypothesis.

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THE IDENTIFICATION OF A NOVEL FACTOR X ACTIVATOR ACTIVITY IN Mg^{2+} - ANTICOAGULATED PLASMA

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Mg^{2+} anticoagulated PPP (20mM $MgCl_2$) was applied at RT to a Zn^{2+} immobilised biscarboxymethylamino Sepharose 4B column and eluted with 20mM Tris, 20mM $MgCl_2$, 2.5mM $CaCl_2$ 0.15M NaCl buffer pH 7.4. Following collection of the wash-through fractions, bound proteins were developed through application of linear (0 to 35mM) imidazole gradients.

All fractions were screened for F.II, V, IX, X, vWF:Ag, Protein C, Fg, Fn and α_2-macroglobulin by ELISA, VIII:Ag by IRMA, and VIII:C by both 1-stage and 2-stage bioassay methods. VIII:Ag (60-90% yield), vWF:Ag (100%), VIII:C 1-stage activity (35%), Fn (> 60%), Fg (> 80%), V (50%) and α_2-macroglobulin (> 70%) were located only in the gradient fractions. Two distinct peaks demonstrated shortening of the 2-stage VIII:C assay:- one co-eluting with the 1-stage VIII:C activity and another major peak in the wash-through fractions where antigenic determinants of F.II, IX, X and part of the protein C were located and partially resolved from each other. Only F.II:Ag co-eluted with the "2-stage VIII:C" activity. Similar observations were found in Mg^{2+} - anticoagulated severe Haemophilia A plasma and in citrated PPP developed with 20mM $MgCl_2$, 20mM Tris buffer. $Al(OH)_3$ treatment abolished the activity from citrate -, but not from Mg^{2+} - anticoagulated plasma.

The relevant fractions showed no activities in F.V, VII, VIII:C, IX or X 1-stage bioassays. They did not clot Fg and did not contain detectable Xa or IIa as assessed by S-2222, S-2238 or S-2288. Following incubation with specific antisera against IgG, II, V, VII:Ag, X, protein C, α- and β- lipoproteins, and plasminogen only anti-II inhibited this "2-stage VIII:C" activity. 2-stage VIII:C assays depend upon Ca^{2+} -dependent generation of Xa. Since the activity in the wash-through fractions could not be ascribed to VIII, Xa, or IIa the results would indicate the presence of a hitherto undescribed Factor X activator activity.

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RAT HEPATOCYTES IN CULTURE INACTIVATE FACTOR Xa. G. D. Qureshi, M. Sun, C. Gervin and H. Evans, Departments of Medicine, Surgery Biochemistry and Pathology, Medical College of Virginia, Richmond, VA.

Plasma contains zymogens of clotting factors, which under various stimuli are activated to serine proteases. Whereas much knowledge has been gained about the activation of clotting factors, relatively little is known about inactivation of these proteases. Antithrombin III has been shown to inactivate some activated clotting factors in plasma. Studies in intact animals have suggested that activated clotting factors are mainly inactivated in the liver. To investigate more fully the role of liver in inactivating the activated factors, we studied the stability of activated factor X(Xa) in hepatocyte cultures. Monolayer cultures on non-proliferating rat hepatocytes were prepared according to the method of Bissell et al. The culture medium was chemically defined and was free from serum or serum products. After the 24 h stabilization period, 0.5 units/ml of 100% activated bovine factor Xa was co-cultured with hepatocytes for 8 h. Samples were collected at 0, ½, 1, 2, 4 and 8 h and tested for Xa activity using chromogenic substrate S-2222. At the end of 8 h only 41.07% of the initial Xa activity remained. Xa inactivation was not affected by a commercially prepared unfractionated heparin (1 unit/ml) and estradiol at 12.5, 25, 125 nM, a potentiator and inhibitor of antithrombin III, respectively. Inactivation of Xa in hepatocyte cultures was inhibited by the addition of cycloheximide (10^{-4}M). Our data suggests that factor Xa is inactivated in hepatocyte cultures by one or more hepatic derived factors which do not meet the functional characteristics of antithrombin III.

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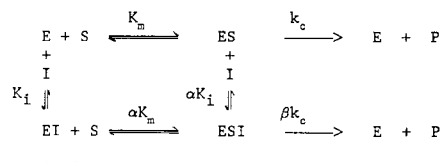
INHIBITION OF HUMAN FACTOR Xa BY ITS ACTIVATOR FROM RUSSELL'S VIPER VENOM. Zbigniew S. Latallo and Craig M. Jackson. American Red Cross Blood Service, S.E. Michigan Region, Detroit, MI, USA

In contrast to bovine Factor Xa, human Factor Xa is inhibited by its activator from Russell's viper venom (X-CP). This inhibition occurs only in the presence of Ca ions. When Xa activity is measured using chromogenic substrates at pH 7.8, inhibition is more apparent at 37°C than 25°C, but can be reduced to nearly zero at high NaCl concentration. The extent of inhibition depends on the source of X-CP. X-CP isolated from *Vipera russelli siamensis*, which activates human Factor X with a K_m = 0.0187 μM and V_{max} of 5.26 x 10^{-11} $M \cdot s^{-1}$ ([X-CP] 0.15 nM, 0.1 M NaCl, 0.01 M TRIS, 0.01 M HEPES, 5mM $CaCl_2$, 0.1% PEG, pH 7.8, 25°C) inhibits the Factor Xa about twice as effectively as X-CP from *Vipera russelli russelli* for which the human Factor X activation rate is about half that observed with V. *russelli siamensis*. The inhibition is of a mixed type and results in doubling the apparent Km value and decreasing V_{max} by 20%. By fitting of the hyperbolic, mixed inhibition model



where E is human Xa, I is *V. russellii siamensis* X-CP, S is Cyclohexylglycyl-glycyl-arginine p-NA, the following values were calculated K_m = 120 μM, k_c = 250 s^{-1}, K_i = 1.0 x 10^{-8}, α = 2.0 and β = 0.82, (0.22 nM Xa, 0.1 M NaCl, 0.01 M TRIS, 0.01 M HEPES, 5 mM $CaCl_2$ 0.1% PEG pH 7.8, 25°C). Activation of human prothrombin by human Factor Xa as well as inhibition of the latter by antithrombin III are similarly affected by the binding of X-CP in the presence of Ca^{++}. X-CP binding to human Xa may explain some of the enigmatic differences in functional properties of bovine and human Factor Xa preparations.

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