504

NORMAL APTT IN A PATIENT WITH A PERSISTENT ACQUIRED FACTOR XI INHIBITOR. R. McKenna (1), E. R. Cole (2), A. Yuk (3) and W. Whisler (4). Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL USA.

A 55 year-old white male (MR) with documented normal hemostatic tests at our institution for 14 years, was admitted for a laminectomy. His pre-operative APTT and prothrombin time activity (Q) were again normal. A prolonged APTT of 58 seconds (21-31 N) and Q of 18% (≥ 70% N) was noted on the 14th post-operative day; patient had received 1 unit PRBC during surgery.

Mixtures of pooled normal plasma (NPP) incubated with MR's plasma (30', 37°C) yielded normal APTTs with as little as 20% NPP in these mixtures, suggesting a plasma factor(s) deficiency. Factor XI level was undetectable with normal values of factors VIII-C, IX and XII. A factor XI level of 7% was found in incubated mixtures (60', 37°C) of equal parts of NPP + MR's plasma as compared to 70% in NPP + buffer and < 1% in MR's native plasma + buffer, indicating the presence of an inhibitor in MR's plasma. Infusion of 1540 ml of FFP (22 ml/kg) to the patient resulted in an APTT of 28 seconds and a Q of 36% with a persistent undetectable XI (expected ∿ 45%). The in-vivo lack of response to FFP was also suggestive of an inhibitor. Additional in-vitro tests showed that the response of MR's plasma was no different from that of NPP with the addition of increasing dilutions of human brain thromboplastin, differentiating this inhibitor from the Lupus type anticoagulant. The severely reduced Q was due to severe temporary reductions in factors II (20%), V (34%), VII/X (2%) and X (20%) without any evidence of hepatocellular dysfunction or vitamin K deficiency. Fibrinogen, thrombin time, FSP, platelet counts and bleeding times remained normal throughout.

There was no external evidence of bleeding after the first operation at any site, despite IM injections and venepunctures. MR was reoperated on day 15 for a cauda equina hematoma; adequate intra and 72 hour post-operative management was with 1 U FFP Q 6 hourly and Amicar; this was associated with a normal PTT, Q ∿35% and undetectable XI, but without generalized bleeding. Discontinuation of this regimen resulted in onset of bleeding from drainage tubes only, without bleeding from the rest of the incision. Reinstitution of 1 U FFP Q 12 hourly and Amicar resulted in a prompt cessation of bleeding. Patient had no evidence for an autoimmune disease, dysproteinemia or any other illness. The potential role of the 1 unit of PRBC transfused cannot be discounted. Further data on this inhibitor will be presented.

505

FACTORS XI AND XII - MICROTITRE AMIDOLYTIC ASSAYS VERSUS CLOTTING ASSAYS. K. Walshe, I.J. Mackie, M. Gallimore, S.J. Machin. Haematology Dept., The Middlesex Hospital, London, W1, UK.

Coagulation assays for FXI and FXII are technically difficult and require expensive human substrate plasmas with a risk of viral contamination: these factors have hampered the study of the contact system in thrombosis and shock. Amidolytic microtitre plate assays have been developed and compared with 1-stage clotting assays. The FXII assay utilises acetone removal of FXIIa inhibitors, blockade of kallikrein and α-2-macroglobulin and S-2222. The FXI assay involves, inactivation of FXI inhibitors, blockade of kallikrein and FXIIa, and measurement of FXI with S-2366. The FXII amidolytic assay was linear up to 200%, and sensitive to <1% FXII, with a cv of 5%. It was not affected by decreased levels of FXI, PKK, or FVII. Normal and deficient patients gave an excellent correlation with clotting assays ($r=0.96$). The normal range (mean +/- 2SD) for FXII was 0.73-1.45 and 0.56-1.48 in amidolytic and clotting assays respectively. Females gave higher values than men in both assay types. The FXI amidolytic assay showed similar linearity, sensitivity and reproducibility and was unaffected by decreased levels of FXII and PKK. 20 normals showed a good correlation with the clotting assay ($r=0.75$), although the latter gave consistently, and significantly higher values ($p<0.05$). The normal range was 0.61-1.41 and 0.60-1.42 in the amidolytic and clotting assays respectively, females again giving higher values than males. Both amidolytic assays are more sensitive, reproducible and economical, using 25% of the reagents for 'tube-type' amidolytic assay, and cheaper reagents than clotting assays. A human substrate plasma is not required, making them safer; large numbers of samples may be rapidly tested. The higher levels of FXI and FXII seen in females may be related to the oral contraceptive pill.

FACTOR XIII

506

THROMBOMODULIN INHIBITS THE ACTIVATION OF FACTOR XIII BY THROMBIN. J. Polgár (1), I. Lérant (2), L. Muszbek (1) and R. Machovich (2). Dept. Clin. Chem., Univ. Sch. Med., Debrecen, Hungary (1) and IInd Inst. Biochem. Semmelweis Univ. Med. Sch., Budapest, Hungary (2).

The thrombogenic functions of thrombin, studied so far, are diminished or blocked when thrombin is bound to the endothelial cell via its receptor protein thrombomodulin. The thrombomodulin-thrombin complex fails to clot fibrinogen, to activate platelet and Factor V, while the activation of the antithrombogenic protein, protein C is extremely enhanced. Although the activation of Factor XIII (FXIII) belongs to the thrombogenic functions of thrombin, the effect of thrombomodulin on this process has not been investigated, so far. The aim of this study was to establish whether the presence of thrombomodulin modifies the effect of thrombin on FXIII. The activation was followed by measuring the transglutaminase activity of FXIIIa formed using our UV-kinetic assay (Muszbek et al., Clin. Chem., 31, 35, 1985) and by monitoring the amount of activation peptide split off by thrombin from the a subunit. The time dependence of FXIII activation using various thrombin concentrations showed significant difference in the presence and absence of thrombomodulin. Thrombomodulin significantly slowed down the activity of thrombin toward FXIII but did not prevent it completely. The possibility that thrombomodulin influences changes brought about by Ca^{2+} and not the action of thrombin was excluded. When thrombomodulin and Ca^{2+} were added only after the proteolytic cleavage of FXIII had taken place, it had no effect on the Ca^{2+} induced activation process. The results suggests that thrombomodulin inhibits the rate of conversion of FXIII to its enzymatically active structure but does not influence the amount of FXIIIa formed.

507

PLASMA FACTOR XIII AND ACTIVATION PROCESS ANALYZED BY ELISA METHOD FOR A2B2 COMPLEX. K. Koike, M. Hada, H. Yorifuji, S. Ikematsu, K. Fukutake and M. Fujimaki. Department of Clinical Pathology, Tokyo Medical College, Tokyo, Japan

Factor XIII induces the crosslinking of fibrin at terminal stage of blood coagulation. New ELISA methods of a-subunit, b-subunit and a2b2 complex of factor XIII were developed by the author and the following results concerning the activation process of factor XIII were obtained. New ELISA methods for a-subunit, b-subunit and a2b2 complex of factor XIII were specific with high sensitivities on each items and indicated the measurement capacity for simultaneous quantitation of many samples, more over we looked upon the dissociation of a2b2 complex with these methods to able to analyze at the subunit levels of factor XIII in details. When a-subunit dimer of platelets was discharged into the plasma by the use of freezing and thawing method on PRP, it was more easily affected to lose their antigeniety than that of a2b2 complex. a-subunit levels in the plasma of congenital factor XIII deficiencies were measured in very low concentration or below the measurement sensitivity of this ELISA. b-subunit levels in the same plasma were indicated around the half of normal levels. These results were as same as another immunological method. It was suggested that the molecular conformation of a-subunit could be changed by the addition of thrombin in high concentration and consequently a-subunit with thrombin was modifyed to show high antigeniety. It was observed that a2b2 complex was dissociated by the addition of thrombin without calcium ion, and the process of this dissociation of a2b2 complex was remarkably accelerated by the addition of calcium ion. Because a-subunit and b-subunit were adsorved on fibrin clot, it might be presumed that the crosslinking of fibrin molecules could be accelerated locally on the surface of fibrin clot.