Thursday, July 16, 1981

Poster Presentations

Coagulation – XVII

Factor XIII

11:00–12:30 h

Grand Ballroom Lobby Boards 257–261

0759

DETECTION AND REGULATION OF THE SUBUNIT PROTEINS OF PLASMA FACTOR XIII. J. McDonagh, C. Skrzynia and S. Ikematsu. Department of Pathology, University of North Carolina School of Medicine, Chapel Hill, U.S.A.

Plasma factor XIII circulates as a noncovalently-associated, tetrameric zymogen (a_2b_2). The a subunit has the potential catalytic function, while the b subunit may act as a carrier protein plasma. In order to study the interactions between the two subunits, specific radioimmunoassays have been developed for each subunit. The assays are valid for measuring a and b protein concentrations in plasma and serum as well as in purified systems. The a assay detects all forms of the protein (zymogen, intermediate, enzyme). Factor XIII activity was measured by the monodansylcadaverine assay. These methods were used to correlate a and b protein concentrations with factor XIII activity in normal donors, in patients with factor XIII deficiency and their family members, and in patients with factor XIII inhibitors.

The normal plasma concentration of each of the subunits of factor XIII is about 12 µg/ml, making the concentration of the zymogen complex to be 0.15 µM. All of the b protein is recovered in serum, while a variable amount of a protein (a*) is found in serum. a protein and factor XIII activity go in parallel in normal, factor XIII deficient, and heterozygous plasma samples. Deficient patients have <1% activity and <20 ng/ml a protein. Deficient patients have about 50% of the normal plasma b concentration, and heterozygotes have 50% a and 75% b protein. In three cases of spontaneous, autoimmune inhibitors against factor XIII, there was no detectable activity and b concentration was one-half normal. Following transfusion of two factor XIII deficient patients with a subunit, activity rose immediately to the expected levels, while b rose more slowly to 20% above the preinfusion level. All of these studies indicate that the circulating level of functional a subunit exerts a positive feedback effect on the concentration of b subunit in plasma.

0760

THE EFFECT OF PLASMIN ON FACTOR XIII (FIBRIN STABILIZING FACTOR). D.M. Rider and J.M. McDonagh. Department of Medicine, School of Medicine, University of Wisconsin, Madison and Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill.

The action of plasmin on several blood clotting factors has been studied; however, controversy exists concerning the effect of plasmin on factor XIII. Factor XIII was purified from plasma and platelets and then exposed to plasmin for up to 6 hours. Plasmin to factor XIII ratios ranged from 0.03-0.1 casein units plasmin per mg factor XIII. These plasmin levels exhibited strong proteolytic activities against B-casein and purified human fibrinogen. Following incubation of factor XIII (activated and unactivated) with plasmin the mixtures were electrophoresed on 7% SDS-polyacrylamide gels. The factor XIII preparations were assayed for ^{14}C-putrescine incorporating activity before and after exposure to plasmin. Platelet factor XIII was labeled with ^{125}Iodine and lableled a subunit (activated and unactivated) was exposed to plasmin for up to 2 hours. These mixtures were electrophoresed on 12.5% Urea-SDS-Polyacrylamide gels and a radioactivity profile was determined for each gel.

Following extensive exposure to plasmin the relative molecular weights of the factor XIII subunits (a, a* and b) remained constant and almost all (90-100%) of the ^{14}C-putrescine incorporating activity was recovered. The radioactivity profiles of the gels of ^{125}I-labeled platelet factor XIII were identical before and after incubation with plasmin. Plasmin did not activate factor XIII in the assay system nor did factor XIII inactivate plasmin by cross-linking it. These experiments indicate that plasmin does not activate or degrade factor XIII and that the b subunit of plasma factor XIII plays no role in protecting the a subunit from the action of plasmin.

0761

EFFECTS OF LEUCOCYTE PROTEASES ON STRUCTURE AND ACTIVITY OF ISOLATED FACTOR XIII SUBUNIT A AND S. F. Holst, H.-G. Klingemann, R. Egbring, H. Bohn, K. Havemann. Center of Internal Medicine, Department of Haematology, University of Marburg and Behringwerke AG, West-Germany.

In some diseases (septicemia, leucemia) an activation of clotting by thrombin (eg. DIC) or an unspecific proteolysis of clotting factors by leucocyte proteases can be responsible for coagulopathy.

We investigated in vitro the influence of elastase-like protease (ELP) and chymotrypsin-like protease (CLP) on isolated purified factor XIII subunit A and S using the SDS-PAA electrophoresis (Weber and Osborn, 1969). ELP and CLP was purified according to W. Schmidt (1974). Factor XIII was purified by Bohn and Haupt.

Results: Using increasing amounts of ELP a correlation between the ELP concentration and the factor XIII split products, which were generated, could be demonstrated. At high concentrations of ELP a complete disappearence of all factor XIII split products could be observed. These digestive effects were shown both on subunit A and on S. The proteolysis of factor XIII was also time dependent. The effects of CLP were not very different from those of ELP. When factor XIII subunit A was digested by ELP, a gradual loss of the ability to crosslink fibrin S occured. As in the literature α_1-antitrypsin strongly and aprotinin slightly inhibited the ELP effects on factor XIII.

Despite the fact that antiprotease potential in vivo is very high, an accelerated turn over of clotting factors can be expected, when ELP forms complexes with its protein substrates like factor XIII. The decrease of factor XIII in some clinical conditions may be caused by leucocyte proteases.