327

ASPIRIN FAILURE TO INHIBIT THE RELEASE OF PLASMINOGEN ACTIVATORS-INHIBITORS BY HUMAN PLATELETS. A.I.Woods,M.A.Lazzari. Department of Haemostasis and Thrombosis,Instituto de Investigaciones Hematológicas,Academia Nacional de Medicina.Buenos Aires,Argentina.

Platelet-PA-Inhibitors can be released by thrombin,Collagen(Col) and others.If they are physiologically important,inhibition of their release might facilitate thrombolysis.Intrinsic PA were tested in euglobulins (eug)of PPP and PPP+Washed platelets(WP),with and without aspirin(ASA),treated with UK,SK and Col(20 and 2ug/ml) Results(mm^2)were:eugPPP:232+78;+3x10^5WP/ul:217+71;+10^6WP/ul:188+75 +2x10^6WP/ul:157+69;With UK:eugPPP:283+76;+3x10^5WP/ul:234+69;+10^6 WP/ul:172+55;+2x10^6WP/ul:154+48; With SK:eugPPP:303+99;+3x10^5WP/ul 252+66;+10^6WP/ul:203+68;+2x10^6WP/ul:174+86;With Col(20ug/ml):eug PPP:234+97;+3x10^5WP/ul:160+63;+10^6WP/ul:141+73;+2x10^6WP/ul:129+81; +2x10^6WP/ul+ASA:105+31;With Col(2ug/ml):eugPPP:230+56;+3x10^5WP/ul: 160+52;+10^6WP/ul:139+44;+2x10^6WP/ul:126+21;+2x10^6WP/ul+ASA:118+28. EugPPP+WP showed lower lysis area.Col induced more decrease of lysis area in eug with WP.ASA did not modify this effect.UK and SK produced higher lysis area only in eugPPP.No difference was observed between high and low doses of Col-effect upon WP.Combined treatment of eugPPP+WP+Col, with UK and SK showed:(mm^2):UK-treated eugPPP+2x10^6WP/ul+Col(20ug/ml):134+25;+ASA and Col(20ug/ml):115+ 65;+Col(2ug/ml):157+50;+ASA and Col(2ug/ml):133+49; SK-treated eugPPP+2x10^6WP/ul:+Col(20ug/ml):144+49;+ASA and Col(20ug/ml):128+ 60;+Col(2ug/ml):173+66;+ASA and Col(2ug/ml):142+36.
Col (20ug/ml) produced slight lower lysis area of UK and SK-treated eugPPP+WP.(p:ns). ASA did not modify this effect.Col(2ug/ml) did not produce changes in lysis area of UK and SK-treated eugPPP +WP. ASA produced slight lower lysis area(p:ns).
Conclusion: high dosis of Col could release anti UK,anti SK and intrinsic PA-inhibitors from platelets;low dosis of Col could only release intrinsic PA-inhibitors.It suggests that anti UK and anti SK release might be triggered by stronger stimuli than intrinsic PA inhibitors.ASA could not inhibit any inhibitors release. We must consider that PA-inhibitors could be released by different metabolic pathways other than cyclooxygenase pathway.

328

COMPARATIVE STUDY OF RADIOIMMUNOASSAYS FOR FRAGMENT E-NEOANTIGEN IN PLASMA AND E-ANTIGEN IN SERUM. J.P. Chen[1], M.H. Goldman[1] and M. Stegnar[2]. University of Tennessee Memorial Research Center and Hospital, Knoxville, TN, U.S.A.[1] and University Clinical Center, Institute of Gerontology, Ljubljana, Yugoslavia[2].

Fragment E-neoantigen (Eneo) is a specific marker of structual and conformational changes associated with the plasmic degradation of fibrinogen and fibrin. The Eneo radioimmunoassay (RIA) is capable of determining normal and pathological plasma levels of fibrin(ogen) degradation products (FDP). However, the detection of the minute quantity of E-related FDP present in the blood of a normal individual requires subjecting the Eneo RIA to a delayed (18 hr) addition of labeled ligand. This step increases the already lengthy period of incubation (48 hr) for the primary antigen-antibody complex to 66 hr. An additional incubation period (24 hr) is required to precipitate the primary complex with goat anti-rabbit IgG.

In an attempt to simplify and speed up the diagnostic blood test for deep vein thrombosis (DVT), the serum fragment E-antigen (E:Ag) RIA has been compared with the Eneo RIA in plasma. Four patients with DVT were followed sequentially with respect to the pre- and post-operative change in response to vascular surgery. Both the Eneo RIA and E:Ag RIAs showed a gradation in plasma (132 - 463 ng/ml) and serum (171 - 1400 ng/ml) E-related FDP levels respectively. Significantly, the sequential measurements of plasma Eneo and serum E:Ag immunoreactivities for these patients appear to fluctuate in parallel with each other. Normal females gave higher plasma Eneo (15.2 ± 4.3 ng/ml) as well as serum E:Ag (42.7 ± 17.8 ng/ml) values than normal males (Eneo = 10.2 ± 5.5 ng/ml, E:Ag = 38.8 ± 19.5 ng/ml). Normal serum E:Ag levels represent the increase in Fg-E concentration, 3-4 times that of normal plasma Eneo levels.

Considerably higher plasma Eneo (2.2 - 7.1 µg/ml of Fg-E equivalent) and serum E:Ag levels were observed in DVT patients under thrombolytic therapy with streptokinase. Western immunoblotting of patients' plasmas and sera revealed that the Fg-E antigenic sites of both pathological plasmas and sera showed a similar pattern with a major immunoreactive FDP at Mr≈360,000. The serum E:Ag assay as currently practiced takes 24 hr to complete, but the time of the assay can be shortened considerably by adapting it to a solid-phase RIA.

329

A NEW QUANTITATIVE ENZYME-IMMUNOASSAY FOR FIBRIN DEGRADATION PRODUCTS (FbDP) IN PLASMA, BASED ON MONOCLONAL ANTIBODIES. P.W. Koppert, E. Hoegee-de Nobel and W. Nieuwenhuizen. Gaubius Institute TNO, Leiden, the Netherlands.

We have developed a sandwich-type enzyme immunoassay (EIA) for the quantitation of fibrin degradation products (FbDP) in plasma with a time-to-result of only 45 minutes. The assay is based on the combination of the specificities of two monoclonal antibodies (FDP-14 and DD-13), developed in our institute. FDP-14, the catching antibody, binds both fibrinogen degradation products (FbgDP) and FbDP. It has its epitope in the E-domain of the fibrinogen molecule on the Bβ-chain between amino acids 54-118 (Blood 68, 437, 1986). Antibody DD-13 was raised using D-dimer as antigen and was used as a tagging antibody, conjugated with horse-radish peroxidase.
A strong positive reaction is obtained with a whole blood clot lysate (lysis induced by tissue-type plasminogen activator) which is used as a standard.
The EIA does not detect FbgDP i.e. purified fragments X, Y, D:E complexes or FbgDP in plasma treated in vitro with streptokinase. This indicates that the assay is specific for fibrin degradation products.
We have successfully applied this assay to the plasma of patients with a variety of diseases. In combination with the assays previously developed by us for FbgDP (Thromb. Haemostas. 1987, in press) and for the total amount (TDP) of FbgDP + FbDP in plasma (J. Lab. Clin. Med. 1987, in press), we are now able to study the composition of TDP in terms of FbgDP and FbDP in patients.