Monday, July 13, 1981

Poster Presentations

Fibrinolysis – III

11:00–12:30 h

Grand Ballroom Lobby Boards 236–242

0183

GENERATION OF FACTOR XII-DEPENDENT PLASMINOGEN ACTIVATOR ACTIVITY IN HUMAN PLASMA MEASURED WITH A FLUOROGENIC SUBSTRATE. G. Dooijewaard and C. Kluft. Gaubius Institute TNO, Leiden, The Netherlands.

A rapid fluorometric assay for measurement of amidolytic activity in human plasma was developed, using the plasminogen activator sensitive synthetic substrate t-BOC-L-valyl--glycyl-L-arginine-β-naphthylamide. The plasma is diluted in a reaction cuvet containing 0.050 M Tris HCl buffer (pH 8.0) and 150 μM substrate. Activation of plasminogen proactivator(s) is initiated at 37°C by the addition of 10 μg dextran sulphate (MW 500,000)/ml. The concentration of β-naphthylamide released is recorded fluorometrically as a function of time. The slope of this curve at any time t is proportional to the concentration of activator. Thus, in a single assay, the entire time-dependent profile of activation and subsequent inhibition is monitored; this provides 1. a value for an optimum plasminogen activator content in the plasma, and 2. the time it takes to reach the optimum. The plot of optimum activator content against μl of plasma added is linear for dilutions more than 100-fold, suggesting that under these conditions the optimum content approaches the content of proactivator(s) originally present.

The activator content measured predominantly consists of contributions of a factor XII-dependent process since 1. without dextran sulphate or with plasmas deficient in factor XII or prekallikrein no activity could be generated, and 2. plots of optimum activator content against dextran sulphate concentration show sigmoidal-shaped saturation curves as found previously for the kallikrein generation in human plasma. Contributions of factor XIIa and kallikrein only partly account for the content measured and studies with plasmas deficient in factor XI point to a minor role for this factor, if any. Further identification of the activator(s) involved is in progress.

0184

ROLE OF THE FIBRINOLYTIC SYSTEM IN RENAL TRANSPLANTATION. P. Glas-Greenwalt and M.H. Goldman. Department of Pathology, University of Cincinnati Medical Center, Cincinnati, OH, and Surgical Service, Veterans Administration, Richmond, Va.

To determine the importance of the fibrinolytic system in renal transplantation on the one hand, and to establish a correlation between possible endothelial damage due to treatment of the renal graft and fibrinolytic parameters on the other, dogs were divided in six groups. Group I dogs were subjected to anesthesia only. Group II was sham operated. In group III, kidneys were perfused with the supernatant of either autologous or homologous cryo-precipitated plasma, and in group IV with albumin. In group V kidneys were cold stored. This was followed by autotransplantation. In group VI kidneys were perfused with albumin and allografted. Before and after transplantation, total plasma plasminogen (pro) activator activities in systemic and renal circulations were measured on fibrin plates after the addition of dextran sulfate and flufenamate to euglobulin fractions. Vascular activator (VA) was measured by adding C1-inactivator. There was no marked difference between groups III, IV and VI. In comparing, however, group V with any of the perfused groups, an overall higher fibrinolytic activity was recorded both for intrinsic activators ($p<.001$) and VA ($P<.001$). In group I a significant drop in both activities ($p<.01$ - $<.02$) could be directly related to the duration of anesthesia with recovery within 24 hours. This pattern, though highly accentuated, revealed itself in all the other groups studied, with VA temporarily reaching zero-levels in the renal circulation in group VI. This data indicates the participation of the fibrinolytic system, in particular of the VA, in determining the fate of renal grafts.

0185

FIBRINOLYTIC PROPERTIES OF MICROBIAL PROTEASES. N.S.Egorov, N.S.Landau, G.V.Andreenko, V.G.Kreyer, S.S.Pokrovskaya, M.A.Karabasova, L.V.Lyutova. Department of Microbiology and Laboratory of Enzymatic Fibrinolysis, Moscow State University, Moscow, USSR

Our study of 820 microorganisms from various systematic and ecological groups revealed that 72% of the cultures contained enzymes capable to dissolve human fibrin clots in vitro. Fibrinolytic enzymes are formed during growth of producents on strictly specific media. Changes in the composition of media and conditions of enzymatic synthesis were found to increase the specificity of microbial proteases to fibrin and to decrease their sensitivity to fibrinolytic enzymes inhibitors. A thrombolytic enzyme (TE) has been isolated and purified from the cultural fluid of actinomycetes. An intravenous injection of TE to albino rats results in an increase of fibrinolytic activity of the blood plasma euglobulin fraction. Time of euglobulin clot lysis is thereby decreased by 32%. The fibrinolytic activity measured from euglobulin precipitate on unheated fibrin plates is increased 2.5-fold. However, the fibrinolytic activity of non-diluted blood plasma remains unchanged probably due to a sharp rise in antiplasmin content following TE injection. TE exerts marked thrombolytic effect in vivo. After intravenous injection of TE to animals with artificial clots in a v.jugularis segment a rapid clot lysis and reconstitution of blood flow are observed.