

Thursday, July 16, 1981

Poster Presentations

Heparin – VI

Fractions, Therapy

11:00–12:30 h

Grand Ballroom Lobby Boards 232–245

INTERACTION OF ATHEROGENIC (VLDL; LDL) AND NON ATHEROGENIC LIPOPROTEINS (HDL) WITH HEPARIN FRACTIONS OF VARIOUS MOLECULAR SIZE. P. Duthilleul, P. Fievet, L. Bouillet, J.C. Fruchart. Laboratoire d'Hématologie. Faculté de Pharmacie -Lille France-. C.T.B. I.N.S.E.R.M. -Lille France-.

Some anticoagulant properties of various heparin fractions (with molecular weights from 5 000 to 20 300 daltons) have been investigated in purified and plasmatic systems with and without lipoproteins isolated by ultracentrifugation from hyperlipemic subjects (type IIa). Amidolytic (Tos-Gly-Pro-Arg-pNA) and coagulometric (Yin and Wessler) anti Xa activity of a HMW fraction (231 USP/mg, MW: 20 300) was decreased from 15 % in both systems supplied with LDL or VLDL since HDL have no effect. No influence was detected on anti IIa activity (Phe-pro-arg-AIE fluorimetric method). LMW (20 USP/mg ; MW 6 000 - 8 000) and ULMW (20 USP.MW 4 000-5 000) anti Xa and anti IIa activities was modified neither by VLDL/LDL neither by HDL. These findings point out to differences in the mechanisms of inhibition of thrombin and Xa by various molecular weigh heparin fractions.

Two dimensional crossed immunoelectrophoresis in agarose 1 % with mixing various quantities of each heparin fraction (0 - 100 µg/ml) in the first phase of electrophoresis of LDL/VLDL, LDL/VLDL and AT III, LDL/VLDL and FXa reveal the presence of LDL or VLDL/heparin fractions complexes and LDL or VLDL/AT III complexes, which are isolated by Gel filtration on Ultrogel A 6. We conclude that FXa can escape from neutralization by inactive complexes.

0737

THROMBIN AND FACTOR Xa INHIBITION BY HOG MUCOSAL HEPARINS FRACTIONATED ACCORDING TO CHARGE DENSITY. M-C. Poon, R.E. Hurst. Departments of Medicine, Public Health and Biochemistry, University of Alabama in Birmingham and Birmingham VA Medical Center, Birmingham, Alabama, U.S.A.

Since heparin inhibition of thrombin (ThI) and factor Xa (XaI) have been shown to depend differently upon the molecular size of heparin, this study was undertaken to determine their dependence upon charge density (Z). Fractions varying systematically in charge density were isolated from 3 hog mucosal heparin preparations (2 decolorized and 1 undecolorized) using a 2 phase sequential extraction system. XaI assay was performed by the plasma clotting method of Yin using a commercial kit. ThI assay involved inhibition of plasma clotting by partially purified bovine thrombin.

When heparin potencies were plotted against Z^2 , all 3 preparations determined by both assays showed a linear relationship with the exception of one fraction with the highest charge density. For each of the 2 assays, the curves for the 2 decolorized preparations were essentially parallel. Although the initial slope of the undecolorized preparation was parallel to those of the decolorized heparins for each assay, the potency of the fraction with highest charge density was different. Thus, all 3 preparations appeared similar for a given anticoagulant function except for the highest charged fractions. When the activity of each preparation was compared to each other, the XaI and ThI curves did not necessarily parallel each other or to the APPT potency curve. No clear cut systematic relationship or differential effects could be established between the heparin anticoagulant functions in relationship to charge density differences. The data suggested also heterogeneity of activities within each fraction with apparent charge density homogeneity.

0738

EFFECTS OF HEPARIN ON THE RATES OF INHIBITION OF THROMBIN AND FACTOR Xa BY ANTITHROMBIN III. M.J. Griffith. Center for Thrombosis and Hemostasis Research. University of North Carolina, Chapel Hill, N.C., USA.

The rate of inhibition of thrombin and Factor Xa by antithrombin III has been investigated as a function of heparin concentration. Three general observations were made. First, the amount of heparin required to maximally enhance the inhibition of thrombin is much less than the amount of heparin required for Factor Xa inhibition by antithrombin III. Second, the maximum rate of thrombin inhibition was approximately 5-fold higher than the maximum rate of Factor Xa inhibition with optimal heparin levels. Third, heparin concentrations greater than 10^{-6} M decreased the rates of inhibition of both Factor Xa and thrombin. Mathematical modeling was attempted to describe the mechanism of action of heparin. At low heparin concentrations ($<10^{-6}$ M) the inhibition of thrombin and Factor Xa could best be described by a model which presumed that binding of heparin to the enzyme accelerates enzyme inhibition by antithrombin III. The effect of heparin at concentrations $>10^{-6}$ on the rates of enzyme inhibition could not be explained by direct binding of heparin to either antithrombin III or thrombin (Factor Xa). This observation has led to the hypothesis that the interaction of thrombin and Factor Xa with heparin is altered at relatively high heparin concentration which results in a decrease in the effectiveness of heparin in accelerating enzyme inhibition by antithrombin III.