

Tuesday, July 14, 1981

Poster Presentations

Heparin – II

Fractions, Analogues, Antithrombin III

11:00–12:30 h

Kent Room Boards 137–148

0349

ANTICOAGULANT ACTIVITY OF HEPARAN SULFATE FROM CEREBRAL MICROVASCULAR TISSUE. J.A. Marcum, L. Fritze and R.D. Rosenberg. Sidney Farber Cancer Institute, Boston, MA., USA.

Microvascular tissue (MT) was isolated by standard sieving techniques from the cerebral cortex of yearling calves. After proteolysis of MT, the cell debris was pelleted and the supernatant was charged to DEAE-Sepharose A-25 equilibrated with 0.1 M NaCl in 0.01 M Tris-HCl, pH 7.5. Uronic acid determination demonstrated that two peaks of mucopolysaccharides (MS) were eluted at an added salt concentration of 0.4 M NaCl and 0.8 M NaCl, respectively. Enzymatic and electrophoretic analyses of the first peak revealed the presence of a minimally sulfated MS with no ability to accelerate thrombin or factor Xa neutralization by antithrombin (AT). MS isolated from the second peak were degraded by chondroitinase ABC lyase and Flavobacterium heparinase. This material migrated slightly behind NIH standard heparan sulfate (HS) and distinctly separately from NIH standard heparin (Hp) which had much greater electrophoretic mobility. Sequential degradation with chondroitinase and heparinase revealed that HS was responsible for the anticoagulant activity of the second peak. The antithrombin and anti-factor X activities of HS were 2.81 u/mg and 2.86 u/mg, respectively. Affinity fractionation of HS with AT resulted in an increase of antithrombin and anti-factor X activities to 36.6 u/mg (13 fold augmentation) and 41.3 u/mg (15 fold augmentation), respectively. Each of the biological activities required the presence of AT and disappeared upon exposure to heparinase. Electrophoretic analysis of the affinity fractionated material demonstrated that it migrated slightly behind NIH standard HS and 40% slower than NIH standard Hp. In conclusion, the ionic strength at which this affinity fractionated HS had been eluted from DEAE-Sepharose and its mobility during electrophoresis clearly distinguish it from classical Hp. These data demonstrate the existence of a specific species of HS from MT with significant anticoagulant activity. These components may in part, be responsible for the non-thrombogenic properties of vascular elements.

0350

INHIBITORY ACTIVITY OF HEPARIN AND OF A HEPARIN SIDE FRACTION (ORG.10172) ON THE ACTIVATION OF THROMBIN IN HUMAN PLASMA. R.Jonker, F.van Houdenhoven, G.van Dedem. Biochemical Research laboratory, Diosynth International, Oss, The Netherlands.

The dynamics of thrombin generation and -inactivation in human plasma was studied in the absence and presence of heparin and of Org 10172, with the purpose of defining (a) mechanism(s) whereby anticoagulant action is accomplished.

Plasma was activated with a standard stimulus of either thromboplastin and Ca^{++} or by kaolin-cephalin and Ca^{++} ; subsamples were taken at regular time intervals and assayed for amidolytic-(S 2238) and antithrombin-3 (AT3) activities. Similar experiments were done in the presence of heparin and of Org 10172.

With heparin, 2 domains could be distinguished: a) a domain (at heparin concentrations below 1 U/ml) where heparin accelerates the inactivation of thrombin and b) a domain (at heparin concentrations above 1 U/ml) where no measurable free thrombin is generated and no measurable drop in AT3 levels could be detected.

With Org 10172 the most pronounced effect was a dose-dependent delay in the onset of free thrombin generation and a weakly dose-dependent reduction in the free thrombin peak levels.

In AT3-free plasma, thrombin generation after a standard stimulus was very rapid and the final constant level of amidolytic activity was higher than in normal plasma. Heparin concentrations of 2 - 4 U/ml changed this pattern to a gradual increase of amidolytic activity to its final level.

Org 10172 showed the same pattern as heparin and also delayed the onset of free thrombin generation in a dose-dependent manner.

It is concluded that a) the anticoagulant effect of heparin in plasma, activated by a standard stimulus is due to at least 2 mechanisms, one of which is probably independent of AT3, and b) Org 10172 exerts its effect independently of AT3, possibly by inhibiting one of the positive feedback loops in the coagulation sequence.