Thursday, July 16, 1981

Oral Presentations

Heparin – III

Fractions, Analogues, Antithrombotic Effects

08:00–09:30 h

Heparin – IV

Antithrombins, Antithrombotic Effects, Antibody

09:45–11:00 h

Dominion Ballroom North

0569

08:00 h

THE ROLE OF HEPARIN IN ANTITHROMBIN III PROTEOLYSIS.
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Recent reports indicate that thrombin is capable of degrading part of the uncomplexed antithrombin III (AT III). Presence of heparin in the reaction of AT III and thrombin, in addition to enhancement of the binding rate, causes an unexplained, excessive decrease in the total AT III binding capacity. This suggests that heparin plays a major role in thrombin-catalyzed AT III proteolysis. Addition of heparin to reaction mixtures of human AT III and α-thrombin, conducted with a large excess of inhibitor, increased the ratio of AT III utilization by more than 50% and induced a release of inactive 50,000-dalton AT III fragment. This fragment, detectable by SDS-polyacrylamide gel electrophoresis with SDS concentrations not exceeding 0.1%, showed a decreased affinity for heparin. It was released from unbound AT III by small quantities of thrombin simultaneously with the formation of AT III-thrombin complex, if at least 1 μg/ml of polydispersed heparin participated in the reaction. To detect a similar degree of proteolysis in heparin-free reaction about 10 times more of α-thrombin was required. The extent of AT III proteolysis promoted by individual heparin fractions obtained in gel filtration correlated inversely with the anticoagulant activity of these fractions. Excessive decrease of residual inhibitory activity and changes in two-dimensional immunoelectrophoresis suggested that part of AT III in plasma is also subjected to proteolysis following addition of thrombin and heparin. These data indicate that the effect of heparin on AT III is more complex than generally recognized. On the one hand, heparin contributes to a rapid neutralization of the enzyme; on the other, heparin facilitates proteolytic degradation of unbound inhibitor even by small quantities of thrombin, causing reduction of the overall binding potential of AT III.

0570

08:15 h

IN VIVO EVALUATION OF THE ANTITHROMBOTIC EFFECTS OF SOME POLYSACCHARIDES OF ULTRA LOW MOLECULAR WEIGHT.
J. Fareed, H. L. Messmore, J. Choay, J. C. Lormeau, M. Petitou, A. Andersen, C. H. Larsen and J. Stulc. Loyola University Medical Center, Maywood, IL., 60153 and Choay Institute, Paris, France.

Ultra low molecular weight saccharide fragments (ULMFs) have been obtained from porcine mucosal heparin (PMH) by extraction (e-ULMF) and by bacterial heparinase depolymerization (h-ULMF-8) processes. Both fragments showed a strong anti Xa activity (>2000 u/mg units, Yin and Wessler, J. Lab. Clin. Med. 81, 298, 1973) and possess relatively weak potencies in the US Pharmacoepial (<40 USP u/mg) and other conventional coagulant assays (activated and non activated partial thromboplastin time, thrombin time and whole blood activated recalcification times). Since ULMFs showed a strong anti Xa activity, we evaluated their antithrombotic actions in a modified stasis-thrombosis model (Wessler et. al. J. App. Phys. 14, 943, 1959) challenging the animals with various thrombogenic stimuli; activated and non activated prothrombin complex concentrates, factor Xa concentrates and human serum. h-ULMF-8 at dosage <0.125 mg/kg (<250 Anti Xa u/kg) IV and <1.0 mg/kg (>2000 anti Xa u/kg) SC completely protected the thrombogenic effects of various thrombogenic agents, whereas PMH at these dosages failed to produce any protection in pre and post treatment regiments. Similar studies with e-ULMF showed protection, however, the antithrombotic rêsponses varied among animals. In vitro supplementation of heparin fragments at 5 times the concentration which protected animals against the thrombogenic effects of activated prothrombin complex concentrates failed to produce any elevation of prothrombin time, partial thromboplastin times, thrombin time and other coagulant assays. Our studies suggest that ULMFs are potent antithrombotic agents and may exert their effects involving multiple sites and primarily inhibiting the Xa and the non-thrombin serine proteases formed during activated states.

0571

08:30 h

A NOVEL ANTI-THROMBOTIC HEPARINOID (ORG 10172) DEVOID OF BLEEDING INDUCING CAPACITY: A SURVEY OF ITS PHARMACOLOGICAL PROPERTIES IN EXPERIMENTAL MODELS IN RATS.
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The pharmacological profile of a heparinoid (Org 10172) was assessed in experimental thrombosis and bleeding models in rats. Org 10172 inhibited thrombus formation in arteriovenous shunts dose dependently (ID_{50} = 5 mg/kg i.v.); heparin USP had an ID_{50} of 0,4 mg/kg i.v. in the same model. Taking the effective dose ratio of the compounds into account both Org 10172 and heparin USP similarly reduced the ^{125}I-fibrin content in thrombi; Org 10172 however affected less the ^{51}Cr-labeled platelet content in the thrombi than heparin USP.

Heparin USP progressively prolonged bleeding at doses of 1 mg/kg i.v. and higher, whereas Org 10172 showed no increase in bleeding over a dose range of 50-200 mg/kg i.v. The benefit (anti-thrombotic)/risk (bleeding) ratio is at least 20-fold higher than for heparin USP.

In vitro Org 10172 did not inhibit the collagen-induced release of serotonin from platelets, in contrast to heparin USP. Org 10172 was at least 500 times less active in enhancing the thrombin-AT III interaction than heparin USP, it showed a very small increase in APTT and had about 6% of the factor X_a-inhibiting activity of heparin USP. The improved profile of the drug may be attributed to these quite different effects on blood platelet function and the coagulation pathway.