

ANTITHROMBOTIC PROPERTIES OF A LOW MOLECULAR WEIGHT HEPARIN. E. Holmer, C. Mattsson, S. Nilsson G. Söderström and C-M Svahn. Research Department, KabiVitrum AB, S-112 87 Stockholm, Sweden.

The ability of heparin to prevent thrombosis has generally been assumed to be reflected by its anti-coagulant activity as measured with clotting assays such as the APTT. Recent clinical studies, however, using a low molecular weight heparin analogue, suggested that this is not necessarily always the case. The analogue had antithrombotic effect although the clotting time was only slightly affected. The present study was undertaken in order to further test this possibility in experimental animals using a low molecular weight heparin fragment with a high relative potency for inhibition of factor Xa. The heparin fragment was obtained after chemical degradation and chromatography on a antithrombin III gel. The molecular weight was 3000-4000 and the specific activity 150 U/mg and 20 U/mg as measured with an anti-Xa based assay and APTT, respectively. The fragment was tested and compared with normal mucosal heparin (corresponding activities 160 U/mg and 165 U/mg) in a rabbit thrombus model as described by Wessler. The method is based on intravenous injection of glass-activated human plasma which induces a transient hypercoagulable state during which a massive thrombus develops in stagnant blood. Thrombus formation were initiated 15 or 30 min after intravenous injection of the different heparins. Two doses, 30 and 60 U/kg (anti-Xa) of both the fragment and ordinary heparin were tested, and the results showed that the fragment was equipotent to heparin in preventing thrombus formation. However, the clotting time as measured with APTT was not significantly prolonged in rabbits treated with the fragment while a two-four fold prolongation was obtained in rabbits treated with ordinary heparin.

## 0357

ANTICOAGULANT EFFECT OF A NEW POTENT HEPARIN IN HUMAN AFTER INTRAVENOUS APPLICATION AND THE COMPARISON WITH COMMERCIALLY AVAILABLE HEPARIN. A.S. Bhargava, H. Wendt and P. Günzel. Research Laboratories, Schering AG, Berlin/Bergkamen (Germany).

The anticoagulant effect of a new potent heparin containing 83% high affinity and 17% low affinity fractions was compared with commercial heparin after a single i.v. application in human subjects. 20 male and 20 female normal adult subjects participated in this study. 2 male and 2 female subjects per group were treated intravenously with doses ranging from 18.3-218.3 µg for new potent heparin and 40.3-483.0 µg for commercial heparin per kg body weight. Determinations of thrombin time, whole blood clotting time, activated partial thromboplastin time and plasma heparin levels using factor Xa inactivation assay were performed before and 5, 10, 15, 30 and 60 min after heparin application. The regression analysis and parallel line assay were performed using log dose or double log transformation or log dose and square root transformation of areas under the curve to estimate the relative potencies of the new heparin preparation.

The new heparin was 1.5 to 2.0 times more effective than commercial heparin per mg dry weight depending upon the coagulation tests used and the length of observation period. These relative potencies of the new heparin in human is in good correlation with relative potencies determined earlier for the same preparation in different in vitro tests including thrombelastography and in vivo using the dog as an experimental animal. In addition, the determinations of biochemical and the haematological parameters, 24h after application indicated no signs of any adverse effect.

## 0356

POSSIBLE MODE OF ACTION OF A SEMI-SYNTHETIC HEPARIN ANALOGUE (SSHA). V.V. Kakkar, B. Djaazaeri, M.F. Scully and J. Westwick. Thrombosis Research Unit, King's College Hospital Medical School, London, England.

It has been suggested that following parenteral injection, the anti-thrombotic effect of SSHA is possibly related to release of endogenous glycosaminoglycans probably from vascular endothelial cell surface. In this study, the mechanism of action of SSHA has been explored further.

Five healthy volunteers received 3 different doses of SSHA (75, 50, 35mg) and 50mg of heparin subcutaneously, four days apart, and venous blood samples were taken at 0,3,5 and 7 hours post injection. These were analysed for prothrombin time, KCCT, lipoprotein lipase, βTG, PF4 and prekallikrein levels. Heparin activity was measured by an anti-factor Xa assay. In addition, template bleeding times were done before and after the administration of 75mg of SSHA and 50mg of heparin. Prothrombin time response, following addition of varying doses of SSHA and heparin to pooled blood was also studied.

Although SSHA prolonged the template bleeding time, the difference as compared with control value was not statistically significant. Heparin in a similar dose prolongs the template bleeding time to a lesser extent. No significant differences were observed in the levels of βTG, lipoprotein lipase, prekallikrein levels, KCCT and heparin activity following administration of SSHA and heparin. Unlike heparin, SSHA in a dose related manner significantly prolongs prothrombin time, the difference being significant at 1,3 and 5 hours following administration of each dose. These findings suggest that part of antithrombotic effect of SSHA may be mediated through inhibition of extrinsic pathway of activation of intravascular coagulation.

## 0358

A MODIFIED STASIS (WESSLER) RABBIT MODEL TO EVALUATE THE ANTITHROMBOTIC ACTIONS OF HEPARIN FRACTIONS AND FRAGMENTS WITH LOW ANTICOAGULANT - HIGH ANTI Xa ACTIVITIES. A. Andersen, J. Fareed, J. Stulc, H.L. Messmore and J. Choay. Loyola University Medical Center, Maywood, IL 60153, USA and Choay Institute, Paris, France.

Since its introduction the Wessler stasis rabbit model (Wessler, et al. J. Appl. Physiol. 14(6), 943, 1959) has been widely used in the evaluation of the thrombogenic properties of prothrombin complex concentrates (activated and non-activated) and antithrombotic effects of various drugs. In order to study the antithrombotic actions of low molecular weight fractions and oligosaccharide fragments we used a modified stasis (rabbit) model with thrombogenic stimuli which produced a marked increase in circulating factor Xa levels. Male New Zealand rabbits ranging from 2-3 kg were injected (subcutaneous) with an analgesic muscle relaxant, xylazine (20 mg/kg) followed by ketamine (80 mg/kg). Prothrombin complex concentrate (PCC) is injected within 2 minutes into a contralateral rabbit ear vein (25 units/kg) immediately followed by Russell's viper venom (RVV). Within varying times after completion of the infusion, (1-15 minutes) previously exposed right and left jugular veins were gently isolated, kept in situ for 10 minutes, carefully excised and the clot formed was graded. Only minor changes were observed in the coagulation profile after the PCC/RVV infusion, however measurable clots in both segments of the isolated vein were seen. Intravenous injection of heparin fractions (<500 µg/kg) and oligosaccharide fragments (<100 µg/kg) blocked the thrombotic effects of PCC/RVV mixture in both the pretreatment and post-treatment regimens, whereas heparin at 500 µg/kg failed to show any antithrombotic effects. Bovine and human factor Xa concentrates, tissue thromboplastins, human serum, human and bovine α-thrombin, and arachidonic have also been employed as thrombogenic stimuli. Our studies show that the modified stasis rabbit model offers definite advantages over the existing model and provides a suitable in vivo standardized model to test antithrombotic effects of newly developed heparin fractions and fragments with high anti Xa and low anticoagulant action.