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**INDUCTION OF OSTEOPOROSIS IN RATS BY STANDARD HEPARIN AND LOW MOLECULAR WEIGHT HEPARIN.** T. Mätzsch*, D. Bergqvist*, U. Hedner**, B. Nilsson***, P. Østergaard**.Depts of Surgery* and Orthopedics***, Malmö General Hospital, Sweden; NOVO A/S Research**, Copenhagen, Denmark.

Long-term treatment with heparin can induce osteoporosis. This complication is suspected to be related to the dosage of heparin rather than to duration of therapy, but the mechanism by which heparin induces osteoporosis is unknown. In a previous study we reported the same degree of reduction in mineral bone mass in rats after treatment with 2 IU heparin/g bw for 33 and 65 days (Thromb Haemostas 1986,56:293-4). Using the same animal model we compared the effect of a high-dose standard heparin (SH) and a low molecular weight heparin (LMWH) in a high and a low dose on the mineral bone mass in the femur of rats.

**Method:** 60 female Wistar rats (207±1.8 g) were randomly allocated to treatment with either 2 XaI U/g bw of standard heparin (SH), 2 XaI U/g bw of LMWH ("high-dose"), 0.4 XaI U/g bw LMWH ("low-dose") or placebo (saline). A standard sodium salt heparin of porcine origin was used, and the LMWH was an enzymatically depolymerized pork mucosal heparin (LHN-1, mean MW 4900 D). Treatment with s.c. injections was continued for 34 days. 24 hours after the last injection the rats were sacrificed and the carefully cleaned femora weighed in air and in water under standardized conditions. Volumes and densities were calculated from the weights. The bones were then incinerated for 48 hours at 600°C and weighed again to determine the ash content (expressed as ash weight per ml of unashed femur volume).

**Results:** There was a significant decrease in ash content ($p<0.01$) and density ($p<0.01$) of the femora in all heparin treated groups as compared with controls. High-dose LMWH caused the same reduction in bone mineral mass as standard heparin. Treatment with low-dose LMWH resulted in a significantly less pronounced reduction in ash content ($p<0.001$) and density ($p<0.05$) of the femora when compared with high-dose standard heparin and high-dose LMWH.

**CONCLUSION:** Daily injections of 2 XaI U/g body weight of standard heparin or low molecular weight heparin for 34 days causes the same degree of reduction of mineral bone mass in rats. The reduction of mineral bone mass in rats by treatment with low molecular weight heparin is dose dependent.

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**HEPARIN IS NOT AN EFFICIENT INHIBITOR OF THE FACTOR Xa-DEPENDENT ACTIVATION OF FACTOR V AND FACTOR VIII.** F.A. Ofosu, G.J. Modi, M.R. Buchanan, J. Hirsh and M.A. Blajchman. Canadian Red Cross Blood Transfusion Service, Depts of Path and Med, McMaster University, Hamilton, Ontario, CANADA.

We have previously proposed that the steps in coagulation most sensitive to inhibition by heparin are the thrombin-dependent activation of factor V and factor VIII. This observation was based on the demonstration that therapeutic concentrations of heparin or 1μM of the thrombin specific inhibitor, phe-pro-arg $CH_2Cl$ (PPACK) completely inhibited the activation of prothrombin when contact-activated plasma (CAP) was recalcified for up to 1 min. Under similar conditions, heparin and PPACK only partially inhibited the activation of factor X. Moreover, the addition of thrombin (10nM) to CAP 1 min before that of heparin or PPACK reversed their inhibitory effects. We now provide further support for our hypothesis by showing that when the activity of thrombin is suppressed by heparin or PPACK, efficient activation of radiolabelled prothrombin occurs only when the factor Xa then present activates factor V and factor VIII. We compared the effects of HEP or PPACK on the following four systems for initiating the activation of prothrombin: (1) CAP; (2) CAP + 10nM thrombin; (3) CAP + 1nM Xa and (4) unactivated plasma + 1nM Xa + 1nM Va + coagulant phospholipids. In each system, the enzymes were added 1 min before the heparin or PPACK. In the absence of heparin or PPACK, all four systems generated the same amount of thrombin activity in 45s. Complete inhibition of prothrombin activation by heparin and PPACK was observed only in system 1 which did not contain exogenous thrombin or factor Xa. No inhibition by heparin or PPACK was observed when thrombin or factor Xa was added to CAP in systems (2) and (3). Only partial inhibition was observed in system (4) which contained exogenous prothrombinase complex. Factor Xa thus provides an effective by-pass mechanism for the activation of factor VIII and factor V in plasma containing therapeutic concentrations of heparin. Our data provide further evidence that the heparin-antithrombin III system is not effective in inactivating factor Xa. These results support the hypothesis that in unactivated normal plasma, the primary anticoagulant effect of heparin is the inhibition of the thrombin-dependent activation of factor V and factor VIII.

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**EFFECT OF $CACL_2$ ON ANTI-Xa ACTIVITIES OF HEPARIN AND LMW HEPARINS.** T.W. Barrowcliffe and Yvonne Le Shirley, National Institute for Biological Standards and Control, Hampstead, London, U.K.

Assays of anti-Xa activity of heparin are generally carried out using plasma or AT III, in the absence of $Ca^{++}$ ions. We studied the effect of physiological (3 mM) $Ca^{++}$ on the relative anti-Xa activities of heparin and LMW heparin, using human AT III, bovine Xa and S-2222.

Potencies of a LMW heparin standard vs. the International Standard (IS) for unfractionated heparin (UFH) were decreased by 45% in the presence of $CaCl_2$. This was shown to be due to a differential effect of $CaCl_2$ on the two substances, with a 93% potentiation in activity of the UFH standard, and only 7% increase for the LMW standard. Addition of $CaCl_2$ to 8 other LMW heparins gave increases in anti-Xa activity ranging from 0 to 60%. Studies on heparin fractions ranging from 4,000 to 30,000 M.Wt., prepared by gel filtration, showed that a minimum M.Wt. of > 6,000 is required for $CaCl_2$ potentiation of anti-Xa activity.

In kinetic studies, the influence of UFH on second order rate constants was increased by 55% in the presence of $CaCl_2$. However, $CaCl_2$ had no effect on the rate enhancement by three high-affinity oligosaccharides, of 5, 10 and 20 saccharide units.

These studies show that the effect of $CaCl_2$ on the anti-Xa activity of heparin is highly dependent on M.Wt. Anti-Xa activities of LMW heparins determined in the absence of $CaCl_2$ could be misleading.

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**A COMPARISON OF DERMATAN SULPHATE AND HEPARIN AS ANTITHROMBOTIC DRUGS.** R.E. Merton, T.W. Barrowcliffe and D.P. Thomas, National Institute for Biological Standards and Control, London, U.K.

Dermatan sulphate (DS) has been shown to accelerate thrombin inhibition by its action on heparin cofactor II (HCII) but has no effect on antithrombin III (Tollefson et al., 1983). In this study, we have examined the in vitro anticoagulant effect of a purified preparation of DS (free from heparin and heparan sulphate), in comparison with that of unfractionated heparin (UFH). We have also studied the effect of DS and UFH in preventing experimental venous thrombosis in rabbits and in inhibiting thrombin generation, both in vitro and in ex vivo plasma samples.

Dermatan had low activity in vitro by APTT and anti-Xa assays (< 5 iu/ mg). When thrombin generation was measured in vitro, 1 μg/ml UFH was sufficient to inhibit thrombin formation. Although 1 μg/ml DS reduced thrombin generation to 40% of control values, there was no further reduction when the concentration of DS was increased to 8 μg/ml.

When DS was injected into rabbits (n = 10), a dose of 150 μg/kg impaired thrombogenesis in a Wessler stasis model. The mean thrombus score was reduced to 25% of the control values, although thrombosis could not be completely prevented, even after an eight-fold increase in dose (1250 μg/kg). When the duration of stasis was extended from 10 to 20 minutes, there was no impairment of thrombosis (mean thrombus score 100%) following 1250 μg/kg of DS. Thrombin generation measured in ex vivo plasma after 150 μg/kg of DS was 72% (s.e.m. 63-81) of that measured in pre-injection plasma. In contrast, 150 μg/kg of heparin prevented thrombosis after both 10 and 20 minutes' stasis (mean score 0%) and thrombin generation was reduced to 17% (s.e.m. 12-23) of control values in ex vivo plasma samples.

Unlike heparin, DS does not completely abolish thrombin generation in vitro and is not as potent as UFH in inhibiting thrombin generation in ex vivo plasma. While DS has demonstrable antithrombotic activity under defined conditions, it is less effective than heparin and increasing doses of DS do not improve antithrombotic effectiveness in this model.