

ANTI-Xa POTENTIATING EFFECT OF LOW MOLECULAR WEIGHT HEPARIN. W. Junker, J. Harenberg, F. Fussi, K. Mattes, R. Zimmermann and E. Weber. Department of Clinical Pharmacology and Internal Medicine, University of Heidelberg, GFR and University of Padua, Italy

Recently special attention has been drawn to bleeding complications of commercial heparins in patients with increased risk for haemorrhages. Alternative heparin preparations with high anti-thrombotic and low haemostaseological properties have been developed. We now report on a new low molecular weight (LMW) heparin (mean MW 5000, 85 USP/mg), which has been obtained by depolymerisation of a heparin from pig intestinal mucosa (mean MW 15000, 154 USP/mg).

In vitro the anti-Xa-activity (chromogenic substrate S2222) was 15% higher for the LMW heparin in a range of 0.01-2.0 USP/ml plasma. No difference was seen on the anti-IIa-activity (thrombin clotting time) and the aPTT for both heparins in the same range. Both Heparins were injected s.c. in a dose of 100, 50 and 25 USP/kg bodyweight into each of six volunteers randomly at weekly intervals. The pharmacodynamic effects were controlled for 6-10 hrs by 8-12 blood samples in relation to the dose applied. Increasing doses the effects of each heparin increased in all test systems. The anti-Xa-activity of LMW heparin was somewhat higher at 100 and 25 USP/kg. At 50 USP/kg the effect of LMW heparin was in the same range as 100 USP/kg of the original preparation (MW 15000). The factor IIa activity and aPTT were not influenced differently by the two heparins at each dose.

The data indicate, that the LMW heparin presented here may have a more pronounced antithrombotic property by a specific anti-Xa-activity than the compared commercial heparin. This effect is most pronounced at doses, which have only small haemostaseological effects.

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ANALYSIS OF CHEMICAL HETEROGENEITY AND PURITY OF COMMERCIAL HEPARINS. R. Hurst, A. Lurie, J. Settle and M. Corum. Departments of Public Health and Pathology, University of Alabama in Birmingham, Birmingham, AL, 35294 USA

The nature of chemical heterogeneity in commercial heparin was investigated, and a practical method of characterization and assay of pharmaceutical heparins was developed. One beef-lung (BL) and six hog-mucosal (HM) heparins were fractionated by sequential extraction of their hexadecylpyridinium complexes in 1-butanol by aqueous solutions containing successively increasing concentrations of NaCl. This system fractionates heparins larger than 10,000 daltons according to their anionic density. The fractions were characterized by molar ratios of carbohydrate constituents and by their anticoagulant potencies in the APTT test with human plasma. Galactosamine content and the amount of uronate extracted with less than 0.14 M NaCl provided a measure of non-heparin GAG and low-quality heparin. The molar ratios of carbohydrate constituents of equivalent fractions from different HM heparins were similar, and with the exception of one unbleached sample, had equivalent anticoagulant potencies which were linearly related to the square of the anionic density. The BL-heparin was clearly distinguishable from the HM-heparins in that its most abundant fractions were extracted at higher NaCl concentrations, and the systematic variation in anionic density seen with HM-heparins was not evident. The fractions were only about half as potent as the equivalent HM-heparin fractions.

A simplified version of the extraction with a two-step partition fractionation was used to measure the percentages of non-heparin GAG and high-quality heparin respectively, and measurements of various molar ratios were used to both characterize the chemical properties of the heparin and its purity. Activation with silica in the APTT test yielded rectilinear semilogarithmic dilution plots whereas ellagic activation yielded curvilinear plots with lower sensitivity.

0740

HEPARIN CHROMATOGRAPHY. R. Losito, H. Gattiker, G. Bilodeau and B. Longpré. Department of Medicine, Centre Hospitalier Universitaire, Sherbrooke, P.Q., Canada.

Commercial heparin is not a homogeneous substance. The isolation of the active component still appears to be a problem and usually requires several different procedures. In order to see if it would be possible to simplify the purification, we studied and compared the chromatography of porcine heparin by a systematic approach using various types of adsorbents, gels and programmed elution techniques employing a Beckman Spectrochrom 130 chromatographic analyzer. A total of 20 combinations were attempted with 200 mg of heparin (169 u/mg) being chromatographed in each case. The fractions of each peak were tested by several methods and included the activated partial thromboplastin time, recalcification time, thrombin time, anti-factor X_a, chromogenic, USP assay, agarose gel electrophoresis and the measurement of metachromatic activity. It was found that dextran gel with an average dry diameter of 75-100 µm produced the best chromatograms whose peaks were well resolved. The medium molecular weight fraction of the second peak contained half of the applied heparin. This peak contained all the anticoagulant activity besides reacting very strongly for metachromatic activity. The smaller and larger molecular fractions found in the other three peaks gave very weak metachromatic activity and were devoid of anticoagulant activity. These results have important implications in the preparation and clinical use of heparin.

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MOLECULAR WEIGHT DETERMINATIONS OF LOW MOLECULAR WEIGHT (LMW) HEPARINS BY ULTRACENTRIFUGATION AND HIGH PRESSURE LIQUID CHROMATOGRAPHY. Grant Barlow, N. Sugisaka and F. J. Petracek. Hematology Unit, University of Rochester School of Medicine, Rochester, NY and Riker Laboratories, 3M Center, St. Paul, MN.

Molecular weights were independently determined on nitrous acid depolymerized LMW heparin fractions ranging from 2-15 daltons using the analytical ultracentrifuge and high pressure liquid chromatography (HPLC).

Sedimentation-diffusion equilibria were obtained in the analytical ultracentrifuge using speeds ranging from 20,000 to 56,000 rpm. Near theta conditions were obtained using 0.5M NaCl as the solvent. Calculations of molecular weight distributions and, from those figures, weight average molecular weights were made using the method described by Scholte (N.Y. Acad Sci. 164, 156, 1969). The results show that weight average values as low as 2,000 daltons can be determined.

The HPLC results were obtained using previously described methods (Fed Proc. 36, 89, 1977) and a new highly efficient gel column (TSK gels). Fractionated dextrans were used as reference standards.

The correlation of weight average molecular weights using these two independent methods was good.