0572

08:45 h

KINETICS OF ANTICOAGULANT AND LIPOLYTIC ACTIVITY AND RADIOLABEL AFTER INTRAVENOUS ADMINISTRATION OF ^{35}S-HEPARIN FRACTIONS OF DIFFERENT MOLECULAR WEIGHT. C.A.M. de Swart, J.J. Sixma, A. Nijmeijer, E. Holmer, L.-O. Andersson and L. Verschoor. Department of Hematology, State University Utrecht, The Netherlands, AB KABI Research, Department of Biochemistry, Stockholm, Sweden and Department of Internal Medicine, State University Hospital "Dijkzigt", Rotterdam, The Netherlands.

Heparin fractions were prepared from commercial pig mucosal by affinity chromatography on antithrombin III, ^{35}S-radiolabelling and gelchromatography. Three fractions were obtained with molwt.of < 6.000, 6 - 35.000 and > 35.000 daltons. They were administered intravenous as a bolus into human volunteers. The anticoagulant activity was measured with the APTT and Xa inactivation, using standard curves. The lipolytic activity was assayed as hepatic triglyceride lipase activity, and as extra-hepatic lipoprotein lipase activity by hydrolysis of tritiated triolein. Radioactivity data were corrected for degraded heparin fragments uncomplexed to protein by subtracting radiolabel with a mol.wt. < 10.000 passing through a filter.

Low mol.wt. fractions induced neither of both lipase activity and had no effect on the APTT. Anti Xa activity and radioactivity disappeared in parallel with slightly concave curves in semilogarthmic plots. Heparin of intermediate and high mol.wt. induced both lipolytic and both anticoagulant activities. The elimination of radioactivity, hepatic triglyceride lipase activity and anti-Xa activity occurred parallel according to a convex curve in semilogarthmic plots. The extra-hepatic lipoprotein lipase activity disappeared following a slightly concave curve.

These data indicate that relatively large heparin molecules are required for lipolytic and APTT activity. Hepatic triglyceride lipase activity might be present complexed to heparin, comparable to the antithrombin III-heparin complex. The elimination of anti Xa activity and hepatic triglyceride lipase activity might be determined by the heparin part of the complexes.

0573

09:00 h

A COMPARATIVE STUDY OF HEPARIN AND HEPARIN FRACTIONS IN PREVENTING EXPERIMENTAL VENOUS THROMBOSIS. D.P. Thomas, T.W. Barrowcliffe, *U. Lindahl, *L. Thunberg, R.E. Merton, K.F. Hiller and C.A. Eggleton. National Institute for Biological Standards and Control, London NW3 6RB and * Swedish University of Agricultural Sciences, S-751 23 Uppsala, Sweden.

We have compared the relative efficacy in preventing venous thrombosis of an ordinary mucosal heparin, a low molecular weight (LMW) heparin fraction and a decasaccharide fragment with high affinity for AT III. We examined the extent to which all three preparations impaired the formation of serum-induced stasis thrombi in New Zealand White rabbits. The LMW fraction, despite having an in vitro potency by APTT half that of ordinary heparin (but comparable anti-Xa activity) was as effective as heparin on a weight basis in preventing thrombosis.

Two minutes after intravenous injection of 30 µg/kg of the LMW fraction the mean blood level by anti-Xa clotting assay was 0.12 i.u./ml (range 0.08-0.21), which was sufficient to prevent thrombosis. In contrast, the decasaccharide fragment, which had a specific activity in vitro by anti-Xa assays of 1000-1300 i.u./mg, but essentially no activity by APTT or thrombin time assays, prevented stasis thrombi only when given at a dose of 100 µg/kg, giving blood levels in excess of 0.3 i.u./ml by anti-Xa assays.

It is concluded that in this experimental model a decasaccharide fragment, despite having a very high affinity for AT III, was less effective on a weight for weight basis than either ordinary heparin or a LMW fraction in preventing venous thrombosis. This suggests that while a sufficiently high anti-Xa activity can alone prevent venous thrombosis, the effectiveness of heparin as an antithrombotic drug does not depend solely on its AT III-binding capacity.

0574

09:15 h

IN VIVO STUDIES ON THE INHIBITION OF COAGULATION BY A HEPARIN ANALOGUE. U. Schmitz-Huebner, F. Asbeck and J. van de Loo. Department of Medicine, University of Muenster, Muenster, W. Germany

SSHA, a semi-synthetic heparin analogue belonging to the chondroitin family, was reported to induce considerable anti-Xa activity in vivo being practically inactive in vitro. In a study designed to elucidate further the in vivo effects of this drug, SSHA and sodium heparin from porcine intestinal mucosa were injected subcutaneously into six volunteers on separate occasions over a period of three days in a cross-over trial. Before injection and 2,4,6,8 hours afterwards, the heparin-like activity was measured by means of the APTT, the anti -Xa clotting test and two chromogenic substrate assays. The results show that SSHA mediates both anti-Xa and antithrombin activities in vivo. A comparison between the effects of SSHA and heparin is problematical, due to the heterogeneity of different heparin preparations. Low doses of the analogue (45 mg s.c.) induce proportionally higher and longer lasting anti-Xa activities than higher doses (90 mg s.c.). In an attempt to identify the mediator involved in the anticoagulant activity induced by SSHA in vivo, antithrombin III (AT III) was removed from a plasma sample of one the subjects obtaining SSHA injections by immunosorption using Sepharose IVb coupled with antibodies against AT III. The AT III free plasma obtained was found to be devoid of heparin-like activity in the anti-Xa clotting test but it maintained its anticoagulant activity in the APTT assay. When purified AT III was added to this plasma its anti-Xa activity was largely restored. In conclusion, the inhibitory effect of SSHA on coagulation seems to involve at least two mechanisms: a direct one which does not depend on AT III and an indirect one, induced in vivo and mediated by AT III.

0575

09:45 h

TURNOVER AND ANTICOAGULANT PROPERTIES OF HEPARIN-ANTITHROMBIN III COMPLEXES, STABILIZED BY COVALENT BONDS. D. Collen, R. Ceustermans, M. De Mol and M. Hoylaerts. Center for Thrombosis and Vascular Research, Department of Medical Research, University of Leuven, Belgium.

High affinity heparin obtained by chromatography of clinical grade heparin on antithrombin III-Sepharose was covalently coupled to antithrombin III using a three step procedure: 1) introduction of free amino groups in the heparin molecule; 2) reaction of these amino groups with the difunctional reagent tolylene-2,4-diisothiocyanate; and 3) reaction of the remaining isothiocyanate group with amino groups of antithrombin III. Amino groups were introduced in the heparin molecule by limited N-desulfation or by reaction of carboxyl groups with hexamethylenediamine with the use of a water soluble carbodiimide. Between 1 and 4 moles of NH_2 groups were introduced per 15,000 g heparin and the yield of the coupling procedures was 25 to 30 percent. The complexes could be separated from free active heparin by chromatography on antithrombin III-Sepharose and from unreacted antithrombin III by gel filtration on a high performance liquid chromatography column. Coupling occurred for 80 percent with an apparent 1:1 stoichiometry. The specific anticoagulant activity of the complexes (expressed per mg heparin) was approximately 75 percent of that of modified heparin and approximately 50 percent of the original high affinity heparin. The half-life of these complexes in blood following intravenous injection of about 100 heparin equivalent units in rabbits was 0.68 $\pm$ 0.08 hours for the N-desulfated heparin-antithrombin III complex and 0.99 $\pm$ 0.27 hours for the hexamethylenediamine substituted heparin-antithrombin III complex which is 2.4 and 3.5 times longer than the half-life of free heparin in the blood. This finding indicates that the main mechanism of disappearance of the anticoagulant activity following intravenous injection of heparin is by removal of free heparin and dissociation of the heparin-antithrombin III complex and not by clearing of the intact complex.