DERMATAN SULFATE IS A MORE POTENT INHIBITOR OF CLOT-BOUND THROMBIN THAN UNFRACTIONATED AND LOW MOLECULAR WEIGHT HEPARINS.

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Clot bound thrombin proteolyses fibrinogen and amplifies the coagulation cascade at its close vicinity, thereby ensuring the growth of fibrin rich thrombus. The present study compares the ability of various glycosaminoglycans (GAGs) to inhibit these 2 properties. Unfractionated heparin (UH), 3 low molecular weight heparins (LMWHs) with increasing antifactor Xa/antifactor IIa ratio, the synthetic pentasaccharide (PS, Sanofi-Organon) devoid of antifactor IIa activity and dermatan sulfate (DS, Mediolanum Farmaceutici) were selected on the basis of their different properties. Proteolysis of fibrinogen by clot-bound thrombin was evaluated by measuring fibrinopeptide A (FPA) generation after an incubation of standardized washed clots in plasma for 120 min in absence or in presence of increasing concentrations of heparins or of DS. The results were compared to those obtained when free α-thrombin (0.4 nM) was added to plasma in the same experimental conditions. On the basis of equivalent antithrombin units UH and LMWHs gave identical results. To inhibit by 70% fibrinogen proteolysis induced by clot-bound thrombin (IC 70) 5 to 9 fold higher concentrations of UH or of LMWHs were required in comparison with those required to inhibit free thrombin. For DS only a 1.3 fold higher concentration was required. PS (final concentration 1 anti Xa U.ml^{-1}) was devoid of any inhibitory effect. The amplification of the coagulation cascade induced by clot-bound thrombin was evaluated by measuring the shortening of whole blood clotting time (WBCT) resulting from the incubation of washed clots into native blood. In absence of GAG, clot-bound thrombin reduced WBCT from 18±2 min to 9±1 min. Each GAG prolonged WBCT in a dose-dependant manner but these prolongations were less important in presence of washed clots. The more potent agent to suppress the shortening of WBCT was DS followed by UH and LMWH. PS (final concentration 1 anti Xa U.ml^{-1}) was almost ineffective. Therefore, in these *in vitro* experiments, DS is a more potent inhibitor of clot-bound thrombin than heparin. Whether or not these observations are relevant for the treatment of established deep vein thrombosis requires comparative clinical studies.

A COMPARATIVE STUDY OF THREE LOW-MOLECULAR WEIGHT HEPARINS (LMWH) AND UNFRACTIONATED HEPARIN (UFH) IN HEALTHY VOLUNTEERS.

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The levels of anti-IIa and anti-Xa activity, as reported in laboratory studies and clinical trials of LMWH preparations, show a high degree of variability. The clinical relevance of anti-IIa and anti-Xa activity is also unclear. The importance of this issue is evident since the incidence of postoperative deep vein thrombosis varies from 8 - 30 % in different LMWH studies on comparable populations undergoing elective hip surgery.

The aim of the study was to compare the in vitro potency of three LMWH preparations and one UFH given as one single subcutaneous injection to healthy volunteers, at the dosage level recommended for orthopedic surgery. The drugs were studied with a controlled, blind and cross-over technique in 12 healthy volunteers. Intra-individual comparison was included in the the design. The following drugs were included: Logiparin (Novo Nordisk A/S) 50 anti-Xa IU/kg b.w., Fragmin (Kabi Pharmacia) 5.000 IU, Klexane (Rhône Poulenc Rorer) 4.000 IU and Heparin (Kabi Pharmacia) 5.000 IU. The following variables were analysed: anti-Factor Xa (Kabi Diagnostica and Stago), anti-Factor IIa (Kabi Diagnostica). Blood samples were drawn from antecubital veins 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 hours after the s.c. injection. All samples were coded and assays were performed under standardized conditions.

	anti-Xa activity (mean±SD)				anti-IIa activity (mean±SD)			
Product	Cmax	t max	AUC	t 1/2	Cmax	t max	AUC	t 1/2
Heparin	0.08±0.05	2.50±0.90	0.67±0.60	non calc.	0.05±0.03	2.64±0.67	0.29±0.21	non calc.
1Fragmin	0.48±0.13	3.00±0.60	2.90±0.73	1.83±0.49	0.09±0.02	3.08±0.79	0.52±0.16	1.05±0.28
2Fragmin	0.49±0.14	3.08±0.90	2.95±0.85	1.78±0.41	0.09±0.04	3.21±0.78	0.50±0.19	1.10±0.31
Klexane	0.42±0.11	3.17±0.58	3.10±0.75	3.55±0.62	0.04±0.01	2.92±0.67	0.20±0.07	1.59±0.93
Logiparin	0.18±0.04	3.08±0.79	0.93±0.29	3.48±0.97	0.05±0.02	3.08±0.52	0.26±0.11	1.49±0.56

Intraindividual variability was small (n.s.) as analysed by the comparison of two identical doses of Fragmin (Fragmin 1 and 2). C_{max}, anti-Xa activity, was significantly lower for Logiparin when compared with Fragmin. t_{max}, anti-Xa activity, did not show any difference between the heparins. The AUC, anti-Xa activity, of Logiparin was significantly lower, as compared with Fragmin and Klexane. $t_{1/2}$, anti-Xa activity, of Klexane was significantly longer as compared with Fragmin and Logiparin.

C_{max}, anti-IIa activity, was significantly higher for Fragmin when compared with Klexane and Logiparin. t_{max}, anti-IIa activity, did not show any difference between the heparins. The AUC, anti-IIa activity, of Fragmin was significantly higher, as compared with Klexane and Logiparin. $t_{1/2}$, anti-IIa activity, did not come out significantly different in a multiple comparison of the heparins. UFH was included in the study as a reference.

This study of three different LMWHs, given as a single injections to human volunteers in the dosages recommended for orthopedic surgery, demonstrates differences between the LMWHs in the potency, expressed as anti-Xa and anti-IIa activity. Klexane showed a longer halflife of anti-Xa activity. Fragmin expressed a higher C_{max} and AUC anti-IIa activity. Whether this difference in inhibition of thrombin has clinical significance remains unclear.

HEPARIN AND ITS INTERACTION WITH ANTITHROMBIN III.

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Heparin is a polydisperse, sulfated linear polysaccharide that has been widely used clinically as an anticoagulant for over a half-century. Heparin's role in anticoagulation involves the regulation of the coagulation cascade primarily through the serine protease inhibitor antithrombin III (ATIII). A decade ago a specific pentasaccharide sequence within heparin, representing the ATIII binding site, was shown to be responsible for heparin's ATIII mediated anticoagulant activity. Detailed structure-activity relationship (SAR) studies on this pentasaccharide have resulted in an understanding of the specific functionality within this pentasaccharide required for the tight binding to ATIII. SAR studies on ATIII have been based primarily on site directed mutagenesis studies. Recently, a theoretical, computer simulation study (Cardin, A.D. & Weintraub, H.J.R., *Arteriosclerosis* 9, 21, 1989) suggested that small linear peptides (consensus peptides, CSPs) are responsible for protein binding to heparin. We have now examined the binding of these peptides to heparin using competitive bioassays and fluorescence studies as well as CD and NMR spectroscopy. These methods demonstrated selective binding of small synthetic CSPs (based on ATIII's sequence) to heparin's ATIII-binding pentasaccharide sequence. Alteration of a single amino acid in the CSP sequence, produces a disrupted consensus peptide (DCSP)), that looses this selectivity. Affinity chromatography using heparin-acrylamide columns showed that DCSP bound with higher affinity than CSP. However, DCSP bound without selectivity to many sites on the heparin polymer; in contrast CSP bound with lower affinity but higher selectivity to the ATIII binding site. The interaction between intact ATIII and heparin has also been examined using a new technique developed in our laboratory based on affinity coelectrophoresis. These studies demonstrate the presence of three types of heparin polysaccharide chains with low, medium and high affinity for ATIII. The molecular weight distribution of chains in each affinity group have also been determined. Affinity coelectrophoresis is also being used to study the placement of ATIII binding sites within the heparin polymer as well as in competitive binding studies between ATIII and CSPs.

HEPARIN CONTENT AND STRUCTURE IN HUMAN HEMANGIOMAS.

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Heparin has been used clinically as an anticoagulant for over a half century; it also has other activities including the ability to regulate angiogenesis. This angiogenic activity is of particular interest since data is presented that describes the isolation, purification and quantitation of heparin from human hemangiomas. Hemangiomas are vascular tumors that correspond to uncontrolled capillary growth. Most of these lesions are infiltrated by 40-fold higher levels of mast cells than found in the surrounding skin. Although patients with hemangiomas have normal clotting times, the blood coming directly from the lesion usually will not clot. The 1-4 pg of heparin found in each mast cell might be responsible for this localized, protamine-nuetralizable, anticoagulant effect. Human heparin (649 µg / g tissue) was recovered and purified from a large (64.5 g wet wt.) hemangioma. Both its chemical structure and *in vitro* anticoagulant activity were characterized. Twelve additional, smaller hemangiomas, ranging from 0.13 to 24.2 g (wet wt.), were also examined for total glycosaminoglycan content by carbazole assay and for heparin content by anti-factor Xa activity. Because of the small size of these tissue samples, the efficiency of glycosaminoglycan extraction and the reproducibility of our analytical method were examined. An average recovery of 61% of the total glycosaminoglycan present was possible by using a commercial protease followed by ion-exchange chromatography and methanol precipitation. An average deviation of 14% and a standard deviation of 35% were obtained (n=5). The glycosaminoglycan content ranged from 0.38 to 3.69 mg/g-tissue and the heparin content ranged from <1 µg to 5 mg/g-tissue. The nearly 10-fold difference observed in glycosaminoglycan content in these hemangiomas, suggest that the high deviation in analysis was acceptable. Neither the mast cell count nor tissue wet weight correlated with either the glycosaminoglycan or the heparin content of these hemangiomas.