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STUDIES ON THE ANTICOAGULANT AND ANTITHROMBOTIC ACTIONS OF DERMATANS AND THEIR SULFATED DERIVATIVES. D. Hoppensteadt (1), A. Kumar (1), J. Fareed (1) and J. Mardigian (2) Dept. of Pathology Loyola Univ. Med. Center, Maywood, Il. (1) Pharmuka Laboratories, Gennivilliers, France (2).

Non-antithrombin III mediated effects such as interaction with heparin cofactor II, modulation of endothelium and polymorphonuclear leukocytes contribute to the overall antithrombotic effects of glycosaminoglycans. In order to study the role of these dermatans, we investigated their in vitro anticoagulant effects using the clot based (PT, APTT, TT, and Heptest), antiprotease (anti IIa and anti Xa) and Thromboplastin C activated fibrinopeptide A generation test. The in vivo antithrombotic actions were investigated, against activated and non activated prothrombin complex concentrates, and in combination with Russells viper venom in jugular and femoral vein stasis thrombosis models (rabbit). The dermatans studied consisted of a standard dermatan of porcine intestinal origin and four sulfated dermatans with varying degrees of sulfation. All of the dermatans studied showed weak anticoagulant effects on the routinely performed clot based assays. Marked variability was seen on the protease inhibition (anti Xa and anti IIa) assays. In the in vivo studies all dermatans studied showed varying degrees of antithrombotic actions against various thrombogenic agents in a modified stasis thrombosis model. Sulfation appeared to produce stronger anticoagulant effects as determined by in vitro assays, whereas the intravenous antithrombotic actions of native dermatan were stronger than sulfated derivatives. This data suggests that dermatans produce their antithrombotic actions via non-antithrombin III mediated pathways. Furthermore, in vitro testing methods are of limited value in the evaluation of the biologic actions of dermatans and their derivatives.

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DERMATAN SULPHATE (DS) INDUCES t-PA RELEASE IN THE PERFUSED RAT HINDQUARTERS. L. Mussoni, M. Abbadini, G.J. Zhu, A. Maggi, J. Pangrazzi, M.B. Donati, Mario Negri Inst., Milano, Italy.

Previous studies have shown that heparin and related glycosaminoglycans (GAGs) were able to induce the release of plasminogen activator (PA) activity in perfusion models. Among GAGs, DS has recently attracted interest due to its antithrombotic effect devoid of haemorrhagic complications in experimental models of venous thrombosis. We report here that highly purified DS (MF-701, Mediolanum Farmaceutici, Milano, Italy) is able to induce t-PA activity release. Non heparinized animals were perfused with oxygenated Tyrode's solution at a constant flow rate of 10 ml/min. PA activity of the perfusate (collected at various time intervals from vena cava) was measured by a fibrin plate method and expressed as mU/ml by comparison with a reference curve of standard t-PA (WHO). Three minutes perfusion of different doses of DS (0.1, 0.2, 0.4 mg/ml) induces a dose-dependent increase in PA activity (from 35 to 60, 99, 127 mU/ml respectively), being maximal during the first and second minute of drug perfusion. A further increase in DS concentration (0.6-0.8 mg/ml) resulted in an inhibition of PA activity released. The PA activity present in the perfusate of animals treated with 0.4 mg/ml DS has been characterized as associated with t-PA-like molecules on the basis of the relative mobility in SDS-PAGE followed by fibrin autography (67,000 m.w.) and inhibition by an antiserum against t-PA in this system. This effect was peculiar for DS, since none of the HMW heparins tested induced PA activity release in this experimental model. These data may suggest that during "in vivo" infusion of DS in a thromboembolic condition some t-PA could be released from the endothelial cells and bind to fibrin in the thrombus to favour its dissolution.

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THE PHARMACOKINETICS OF ^{125}-I DERMATAN SULFATE IN THE RABBIT. **F. Dol (1), G. Houin (2), D. Dupouy (1), C. Caranobe (1), Y. Cadroy (1), P. Sié (1) and B. Boneu (1). Laboratoire d'Hémostase, Centre de Transfusion Sanguine (1) and Laboratoire de Pharmacocinétique, Hôpital Purpan (2) 31059 Toulouse cedex, FRANCE.**

We have determined the main pharmacokinetic parameters of dermatan sulfate (DS), a catalyst of IIa-heparin cofactor II (HC II) interaction which presents antithrombotic properties in the rabbit. DS (Pharmuka, France) was conjugated with SHPP and iodinated using the chloramine T method. The labelled derivative had the same MW distribution and biological activities than the native one. Rabbits were injected by 5 ucies of ^{125}I-DS (0.6 ug) and increasing doses of unlabelled DS. Serial blood samples were collected to measure cpm disappearance and, in some cases, residual biological activity was determined (ex vivo quantitation of IIa-^{125}I-HC II complexes). The cpm curves were broken into 3 exponentials : alpha, beta and gamma. The beta exponential was closely superimposable to the curves of biological activity disappearance. The main pharmacokinetic parameters are indicated in the Table (mean ± SD) : there was a slight (non-significant) tendency to the half life (T1/2) prolongation and to the reduction of both the clearance (cl) and the volume of distribution (Vd). Thus after IV injection, the pharmacokinetics of DS mimics that of LMW-heparin in the rabbit : T1/2 is in the same order of magnitude and independent of the dose delivered. These results are promising for the future development of this compound as an antithrombotic agent.

Dose ug/kg	n	T 1/2 min	cl ml/min	Vd ml/kg
20	5	12.4 ± 2.4	13.4 ± 2.3	106 ± 33
200	4	12.7 ± 2.1	12.6 ± 3.1	100 ± 19
2000	5	13.8 ± 2.1	10.6 ± 3.6	87 ± 19
4000	4	13.4 ± 0.7	9.5 ± 1.5	79 ± 36

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KINETICS OF EXPERIMENTAL ANTITHROMBOTIC ACTIVITY OF MF 701 DERMATAN SULFATE. A. Morani (1), F. Gianese (2) and P. Bianchini (1) Laboratorio Gamma, Modena, Italy and Medical Department, Mediolanum Farmaceutici, Milan, Italy (2).

In order to evaluate the intensity and duration of the prophylactic activity of dermatan sulfate against thrombosis induced by ligature of the inferior vena cava (Reyers'model), we treated 6 groups of rats at increasing intervals of time before ligature (1 min, 30 min, 45 min, 2 h, 16 h). Within each group the rats were injected subcutaneously with saline solution (controls) or increasing doses (2.5, 5, 10 and 20 mg/kg) of MF 701, dermatan sulfate (Mediolanum Farmaceutici, Milan, Italy). Two hours after ligature the presence of thrombi and their weight were recorded.

A significant, dose-dependent antithrombotic effect was observed for all the treatment times. The maximum effect occured when the compound was administered 45 min before ligature (total abolition of thrombosis at the dose of 5 or more mg/kg). Total inhibition was also observed at 2 h (with 10 mg/kg) and at 4 h (with the same dose). A significant antithrombotic effect, over 50% compared with the controls, was still found at 16 h at the dose of 20 mg/kg.

These results confirm the experimental antithrombotic efficacy of dermatan sulfate already reported in a different animal model by F. Fernandez et al. (Br J. Haematol. 64, 309, 1986) and demonstrate the long duration of the prophylactic effect of the compound.