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REGULAR ARTICLE

Antithrombotic and anticomplementary properties of a very low molecular mass dermatan sulfate

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Abstract

Introduction: Dermatan sulfate, a sulfated glycosaminoglycan, acts as an anticoagulant by accelerating the inhibition of thrombin by heparin cofactor II.
Materials and methods: A low molecular mass dermatan sulfate was obtained by peroxy-radical depolymerization from a parent dermatan sulfate. Chemical characterization of this low molecular mass dermatan sulfate shows a material of ~ 5 kDa that conserves sulfated sequences (2-*O*-sulfation of the iduronic acid units and/or 4 or 6 positions of galactosamina *N* acetyl) essential for dermatan sulfate–heparin cofactor II interaction with more sulphated proportion (27.7 ± 1.9 μg% *vs* 11.5 ± 0.8 μg%, $P < 0.05$ $n = 6$, low molecular mass dermatan sulfate *vs* dermatan sulfate).
Results: After a single intravenous administration of low molecular dermatan sulfate in rats, fibrinolytic activity increased simultaneously with thrombin clotting time prolongation. Low molecular dermatan sulfate showed an inhibitory effect on classical complement activation pathway reaching a maximum during the first hour. Furthermore, low molecular dermatan sulfate was as effective as dermatan sulfate to prevent thrombus formation and to diminish thrombus weight in a rat venous thrombosis model.

Abbreviations: C1, first protein complex; DS, Dermatan sulfate; EA, antibody-sensitized sheep antibody-opsonized erythrocytes; EF, euglobulin fraction; HCII, heparin cofactor II; IV, intravenous administration; LMMDS, low molecular mass dermatan sulfate; LMWH, low molecular weight heparin; PAGE, polyacrylamide gel electrophoresis; RPS, rat pool serum; TAFI, thrombin activatable fibrinolysis inhibitor; T, thrombin; TCT, thrombin clotting time; t-PA, tissular plasminogen activator; UFH, unfractionated heparin.

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Conclusions: The results indicate that peroxy-radical depolymerization of dermatan sulfate produced a low molecular dermatan sulfate with profibrinolytic, thrombolytic, antithrombotic and anticomplement properties. We conclude that low molecular dermatan sulfate may be an effective adjunct in the management of thrombotic events.
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Dermatan sulfate (DS) is largely distributed in connective tissues of mammals, other vertebrates and marine invertebrates [1,2].

DS shows antithrombinic activity, catalyzing heparin cofactor II (HCII) [3], a selective thrombin (T) inhibitor. This activity has been found effective in the prevention and treatment of arterial and deep vein thrombosis with a low rate of hemorrhagic complications [4–7].

DS is capable of inhibiting free and fibrin-bound thrombin, unlike unfractionated heparin (UFH) and low molecular weight heparin (LMWH) which only act over free T [8,9]. Besides, DS induces a significant release of tissue plasminogen activator (t-PA) as a possible direct effect on endothelial cells [10].

Previous *in vitro* studies provide suggestive evidence showing that DS can inhibit the enzymatic activities of several complement components [11]. Nevertheless, the mechanisms that may contribute to these inhibitory properties are not clearly understood.

Our research group has described the existence of a very small subpopulation of low molecular mass DS, isolated by precipitation with the first protein complex of human complement system (C1) [12]. This precipitated subpopulation of DS increases its antithrombinic and thrombolytic activities with a concomitant amount of the sulfate content.

A low molecular mass dermatan sulfate (LMMDS) with similar chemical characteristics can be obtained by peroxy-radical depolymerization. This chemical treatment decreases the molecular mass, enhances sulfation and slightly alters the digestibility by chondroitinase ABC [13].

The present study was designed to evaluate the biological activities of LMMDS obtained by peroxy-radical depolymerization. Particularly, its activities over anticoagulant and fibrinolytic systems, and over the classical complement activation pathway, in all cases after intravenous administration (IV). Furthermore, we have evaluated its ability to reduce or prevent occlusive thrombus formation in rat model.

Materials and methods

Materials

DS and LMMDS were a gift by Syntex S.A., Argentina. DS was extracted from bovine intestinal mucosa, (batch No. 082-91). LMMDS was obtained by peroxy-radical molecular depolymerization from DS (US Patent 4977250 and Eur. Patent 26888).

Glycosaminoglycans molecular weight markers, heparin ammonium salt (UFH) 179.3 units/mg (H-0880), IgG–agarose (A-6284), human thrombin (T-6759), fibrinogen fraction I from bovine plasma (F-4753) were purchased from Sigma Chem. (St. Louis, USA). Protein standard weight markers were from Bio-Rad Labs (Richmond, Canada). Heparin standard was from INAME, Argentina. General reagents were of analytical grade or higher.

Animals

The procedures used in this study are in accordance with the guidelines of Facultad de Farmacia y Bioquímica Committee on the use and care of animals in agreement with the Institutional Use Review Board of the Universidad de Buenos Aires. This study was carried out on male Wistar rats weighing 220–250 g. Veterinary care was provided by the breeding laboratories of Facultad de Farmacia y Bioquímica (Universidad de Buenos Aires, Argentina).

In vitro studies

Chemical characterization of low molecular mass dermatan sulfate.

The molecular mass of DS and LMMDS was estimated by 6% polyacrylamide gel electrophoresis (PAGE) [2]. High molecular dextran sulfate (average molecular mass = 500 kDa), chondroitin-6-sulfate from shark cartilage (average molecular mass = 60 kDa), and low molecular weight dextran sulfate (average molecular mass = 5 kDa) were used as molecular mass markers.

^{13}C NMR spectroscopy and agarose gel electrophoresis were analyzed as it was described [14,15].

The sulfate concentration was determined by the sodium rodizonate method after desalting samples [12]. The *in vitro* potency of LMMDS and DS was standarized against United States Pharmacopoeia (USP) heparin reference standard (INAME), Argentina, using USP assay and human plasma.

Bioavailability assays and *in vivo* studies of biological activities of dermatan sulfate populations

For bioavailability studies, 0.3 mg kg^{-1} of DS starting material or 0.2 mg kg^{-1} of LMMDS was administered to animals into the dorsal vein of the penis, employing physiological solution as a vehicle.

After administration, 9 volumes of blood were collected, at several times, by direct intracardiac puncture and mixed with 1 volume of 3.8% trisodium citrate for thrombin clotting time, (TCT) or acidic disodium citrate for fibrinolytic assay, into polystyrene tubes. Samples were centrifuged within 30 min at 2000 g for 15 min. TCT was performed within 2 h following blood collection. Samples destined to fibrinolytic assay were frozen and kept at − 70 °C until they were used. TCT was determined employing a solution of human T at 1 NIH U/ml by standard

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