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REGULAR ARTICLE

Effect of oversulfated dermatan sulfate derivatives on platelet aggregation

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Abstract We have investigated the effect on human platelet aggregation of native dermatan sulfate (DS) and three oversulfated DS derivatives with different sulfur contents, and compared it with that of unfractionated heparin. An inhibitory effect on collagen-induced platelet aggregation was observed only with unfractionated heparin at high concentrations, whereas no inhibitory effect was observed when arachidonic acid was used. Heparin was the most potent inhibitor of the thrombin-induced platelet aggregation in platelet-rich plasma (PRP), whereas the oversulfated DS had a higher potency than the native DS. All these glycosaminoglycans (GAGs) also inhibited thrombin-induced aggregation of washed platelets in the presence of antithrombin (AT) or heparin cofactor II (HCII) but not in their absence. Heparin was by far the most potent inhibitor of washed platelet aggregation in the presence of AT, whereas the inhibitory effects of the DS (native or oversulfated) were lower but dependent on the sulfur content. In the presence of HCII, DSb, a slightly oversulfated DS, had the highest inhibitory effect, whereas heparin and DSd, the most oversulfated derivative, had lower potencies in this case. These data suggest that the inhibition of thrombin-induced platelet aggregation by the oversulfated DS derivatives is related to their ability to potentiate thrombin inactivation by AT or HCII. Hence, the

The anticoagulant activity of dermatan sulfate (DS) is improved when its level of sulfation is increased [1,2]. We reported previously that the anticoagulant efficacy of oversulfated DS depends on both the degree of sulfation and the oversulfation procedure

Abbreviations: DS, dermatan sulfate; GAGs, glycosaminoglycans; AT, antithrombin; HCII, heparin cofactor II, GlcA, glucuronic acid; IdoA, iduronic acid; GalNAc, *N*-acetyl-galactosamine.

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oversulfated DS derivatives may not have an effect per se on the inhibition of platelet aggregation. They may constitute a new class of anticoagulants with enhanced anticoagulant effects in comparison with the native DS, but with only minor side-effects of bleeding in comparison with heparin.

[2]. Indeed, oversulfation enhances the ability of DS to accelerate the thrombin inactivation by heparin cofactor II (HCII) [3] but also by antithrombin (AT) [4].

However, the clinical use of these glycosaminoglycans (GAGs) is associated with side-effects of bleeding [5]. Indeed, heparin and various GAGs have been shown to affect platelet function [6]. Moreover, because bleeding and heparin-induced thrombocytopenia [7] represent major side-effects of heparin, which remains the most widely used anticoagulant drug [8], alternative drugs are being sought [9,10].

In this study we have investigated the effect on human platelet aggregation of a natural DS and three DS derivatives of varying sulfur content, obtained by two different methods of oversulfation, compared to that of unfractionated heparin. The inhibiting effect of these GAGs was studied on platelet-rich plasma (PRP) after platelet aggregation induced by collagen, arachidonic acid and thrombin, and on washed platelets with thrombin as agonist.

Materials and methods

Materials

All the dermatan sulfate derivatives were supplied by Rhone Poulenc Santé, Gennevilliers, France: natural dermatan sulfate from bovine intestinal mucosa (DSa, 47 kDa, sulfur content (S) 6.0%). The three other oversulfated dermatan sulfates (mean molecular weights DSb, 50 kDa, DSc, 50 kDa, and DSd, 51.2 kDa) were obtained by two different methods of oversulfation, leading to different sulfur contents as previously described: in method A, formamide was used to dissolve the DS sodium salt before the addition of the SO_3-trimethylamine complex, whereas in method B the DS benzethonium salt was dissolved in dimethyl formamide before the addition of SO_3-trimethylamine [2]. The characteristics of the DS derivatives are summarized in Table 1.

Unfractionated standard heparin (150 IU/mg, 15,000 g/mol), was from LEO, Montigny-Le-Bretonneux, France). Human heparin cofactor II (HCII, 10 U/mg) was purified as previously described [11]. Purified antithrombin (AT, 5 U/mg) was purchased from Kabivitrum, Stockholm, Sweden. Soluble collagen was from Organon Teknika Corporation, Durham, North Carolina, USA. Purified human thrombin (3200 NIH U/mg), arachidonic acid (Na-), PGE1 and apyrase were from Sigma Chemical Co., St. Louis, USA. Human fibrinogen was prepared as previously described [12].

Methods

Blood was obtained from 20 healthy volunteers, aged 20-40 years, who denied taking drugs which interfere with platelet function. Blood (9 vol) was directly drawn from the cubital vein into trisodium citrate 0.13 M (1 vol). Platelet-rich plasma (PRP) was prepared by blood centrifugation at 200 *g* for 10 min (20 °C). Aggregation tests were performed at 37 °C within 3 h after the venipuncture.

Platelet aggregation in PRP was measured by a turbidimetric method (final platelet count was 300,000/μL) on a Chronolog 550 (Coultronics, Margency, France). Baseline (0%) and 100% aggregation were established by measuring the light transmission through PRP and platelet-poor plasma (PPP) respectively. GAGs at various concentrations were added to 400 μL of PRP; after 1 min of incubation at 37 °C, thrombin (final concentration (fc) 0.3 U/mL) was added and the aggregation recorded for 15 min. The same procedure was performed when using collagen (fc 0.3, 0.5 and 0.75 μg/mL) and arachidonic acid (0.25, 0.5 and 0.85 mM) in three sets of experiments. These concentrations were the lowest concentrations of collagen and arachidonic acid, respectively, which induce complete platelet aggregation in the three sets of experiments. Controls without GAGs were performed at the same time.

Washed platelets were prepared as previously described [13]. Blood (50 mL) was drawn in 9 mL of a solution of ACD containing apyrase (25 mg/L) and

Table 1 Some characteristics of the DS used, native or oversulfated

Characteristics	DSa	DSb	DSc	DSd
Origin	Bovine intestinal mucosa	Bovine intestinal mucosa	Porcine skin	Bovine intestinal mucosa
Mean molecular weight	47 kDa	50 kDa	50 kDa	51.2 kDa
Sulfur content, %	6.0	7.8	9.0	11.5
Oversulfation procedure	None	A	B	B