Br. J. Pharmac. (1980), **69**, 675–677

ANTICOAGULANT EFFECTS OF SULPHATED POLYSACCHARIDES IN NORMAL AND ANTITHROMBIN III-DEFICIENT PLASMAS

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1 Comparison of the effects of sulphated polysaccharides on thrombin-induced clotting of normal and antithrombin III-deficient plasmas suggests the involvement of antithrombin III (AT III) in the anticoagulant activities of cellulose, dextran and xylan sulphates.

2 AT III appears to play little or no role in the anticoagulant activity of carrageenans.

Introduction

Several sulphated polysaccharides exhibit anticoagulant properties. Cellulose sulphate and dextran sulphate are among many artificially-substituted polymers assessed as anticoagulants because of their general chemical resemblance to heparins (Astrup, Galsmar & Volkert, 1944); the sulphated xylan SP54 has been used clinically to reduce thrombogenesis (Wade, 1977); and the potential as immunosuppressive and antipeptic agents of carrageenans, the algal galactan sulphates, is complicated by their anticoagulant activities *in vivo* (Hawkins & Leonard, 1962; Everson, Stacey & Bell, 1977).

Anticoagulant activity of heparins (Barrowcliffe, Johnson & Thomas, 1978), and of at least some other naturally-occurring sulphated glycosaminoglycans (Teien, Abildgaard & Höök, 1976; Kindness, Long & Williamson, 1979a), appears to involve potentiation of the antiprotease antithrombin III (AT III), an inhibitor of thrombin and several other blood serine proteases which catalyse particular steps of the coagulation pathway. In this paper we assess the role of AT III in the *ex vivo* anticoagulant activities of cellulose, dextran, xylan and galactan sulphates, using plasmas genetically-deficient in AT III.

Methods

Preparation of plasmas

A pool of normal human plasma from 20 donors was prepared as described previously (Kindness, Long & Williamson, 1980a). Citrated plasma from a woman (subject A) and a man (Subject B) with familial AT III deficiences (Mackie, Bennett, Ogston & Douglas, 1978) was also prepared. Both subjects were being routinely maintained on long-term Warfarin therapy, and displayed no clinical or laboratory evidence of recent thrombosis or liver disease. Their blood concentrations of α_1-antitrypsin and α_2-macroglobulin were normal. Concentrations of AT III were measured by functional assay (Abildgaard, Gravem & Godal, 1970) and by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) on commercially-available plates (Behringwerke AG, Marburg, F.D.R.). AT III concentrations (as % of that present in normal pooled plasma) were: subject A 45% (functional), 47% (immunological); subject B 51% (functional), 57% (immunological).

Measurement of thrombin times

The method of Ratnoff (1954) was used. Thrombin times (mean ± s.d.) were obtained from quadruplicate results; coefficients of variation ranged from 6 to 12. Results were expressed as clotting ratios (thrombin time in presence of anticoagulant/thrombin time in absence of anticoagulant). Control clotting times (in absence of anticoagulant) were in the range 12 to 15 s for all plasma samples. If no clot formed in anticoagulant-containing tubes after 120 s, the clotting ratio was recorded as infinity.

Materials

The sources, molecular properties and methods of preparation of cellulose sulphate, heparin and dermatan sulphate (Kindness *et al.*, 1980a), dextran sulphate (Kindness, Williamson & Long, 1979d), xylan sulphate, SP54 (Kindness *et al.*, 1979e) and λ-carrageenan (Kindness, Long, Williamson & Boyd, 1979c) were as previously described.

0007-1188/80/080675-03 $01.00

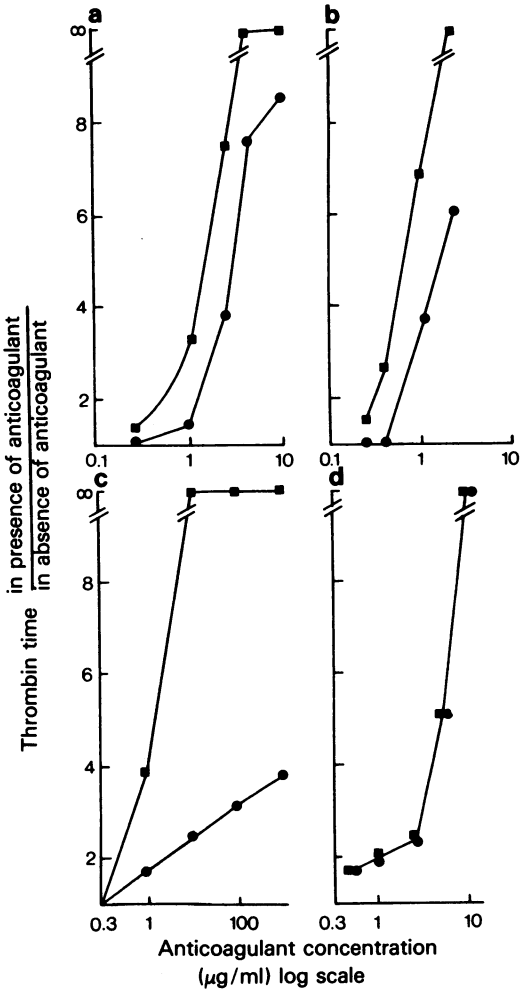

Figure 1 Effect of anticoagulants on thrombin times of normal pooled plasma (■) and AT III-deficient plasma (●). (a) dextran sulphate; (b) cellulose sulphate; (c) xylan sulphate; (d) λ-carrageenan. Subject A plasma was used in (a); in other experiments subject B plasma was used.

Results

In a preliminary experiment, the effects of heparin (0.33 μg/ml) and dermatan sulphate (100 μg/ml) on normal and AT III-deficient plasmas were investigated. Clotting ratios (see Methods) recorded were: normal pooled plasma, heparin ∞, dermatan sulphate 2.6; subject A plasma, heparin 2.0, dermatan sulphate 2.6; subject B plasma, heparin 3.0, dermatan sulphate 2.7.

Figure 1 shows that, over a range of concentrations, the anticoagulant activities of cellulose, dextran and xylan sulphates were, like that of heparin, reduced in AT III-deficient plasma. The anticoagulant activity of λ-carrageenan, like that of dermatan sulphate, was not reduced in the deficient plasma.

Discussion

Heparin and dermatan sulphate were included as reference compounds in these experiments because of the known involvement of AT III in the activity of heparin, and possible lack of involvement of AT III in the activity of dermatan sulphate (Teien *et al.*, 1976; Kindness *et al.*, 1979a). The reduced *ex vivo* anticoagulant activity of heparin in AT III-deficient plasma, was increased in plasma taken immediately after administration of AT III to an AT III-deficient patient (Kindness, Williamson & Long, 1980b).

Like heparin, cellulose, dextran, galactan and xylan sulphates potentiate AT III inhibition of the hydrolysis of chromogenic amides by purified thrombin and factor X_a (Czapek, Kwaan, Szczecinski & Friedman, 1978; Kindness *et al.*, 1979a, d, e; 1980a). Moreover, the *ex vivo* anticoagulant activity of cellulose, dextran and xylan sulphates is, like that of heparin, reduced following preincubation of plasma with antiserum specific for human AT III; the activity of carrageenans is not reduced (Kindness *et al.*, 1979b). These results, which are in accordance with those described here, suggest that AT III is involved in the anticoagulant activity of cellulose, dextran and xylan sulphates, but plays a minor, or no role in the activity of carrageenans. Galactan and xylan sulphates also resemble heparin in inducing an *ex vivo* aggregation of human platelets which is prevented by preincubation of platelets with AT III (Kindness *et al.*, 1979c, f; 1980b), and in acting synergistically with AT III to inhibit thrombin-induced aggregation of platelets (Kindness, Williamson & Long, 1980c).

The molecular features of sulphated glycosaminoglycans important for AT III activation may involve critical polymer size, particular patterns of ionic substitutions, and the degree of polymer rigidity (Rosenberg, Armand & Lam, 1978; Boyd, Long & Williamson, 1979; Lindahl, Backström, Höök, Riesenfeld & Thunberg, 1979). Further work on sulphated polysaccharides of well-defined chemical structures should help clarify these features.

We thank Professor H.M. Keir and Professor A.S. Douglas for their interest and support, Dr N.B. Bennett and Dr J.H. Winter for their helpful advice, and for providing the AT III-deficient plasma samples, and Benechemie GMBH for providing the SP54 samples.

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(Received September 6, 1979.
Revised November 2, 1979.)