Published in final edited form as:

Thromb Haemost. 2008 July ; 100(1): 160–162. doi:10.1160/TH08-05-0275.

Heparin is procoagulant in the absence of antithrombin

Stephanie A. Smith¹ and James H. Morrissey²

¹Department of Internal Medicine, College of Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

²Department of Biochemistry, College of Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

Dear Sir,

Antithrombin is a serine protease inhibitor that inactivates thrombin, factor Xa (FXa) and other coagulation proteases. The inhibitory activity of AT is enhanced many fold by heparin or endothelial cell surface heparan sulfate proteoglycans (HSPGs) (1) and a variety of non-human origin proteoglycans exhibit anticoagulant activity (2,3). Approximately 30% of heparin molecules in pharmaceutical preparations of unfractionated heparin (UFH) contain a specific sulfated pentasaccharide sequence that confers high affinity binding to AT (1), but less than 5% of endothelial HSPG molecules bind AT (5,6). Heparins and HSPGs are highly anionic and they bind to many other proteins, including growth factors, extracellular matrix proteins, selectins, lipoproteins, cytokines and chemokines. As a result, HPSGs have a wide variety of nonanticoagulant effects, including influences on platelet function, cell adhesion, inflammation, apoptosis, angiogenesis, and development (1,4).

A recent report by Liu et al. (8) described the procoagulant activity and ability to abrogate tissue factor pathway inhibitor (TFPI) function by AV513, a non-anticoagulant sulfated proteoglycan (NASP) of plant origin. Studies in our laboratory suggest that AV513 accelerates thrombin generation through promoting the activation of factor V (FV) (9). We also recently reported that polyphosphate (polyP), a linear polymer of inorganic phosphate (10), is a potent procoagulant (11). PolyP accelerates blood coagulation by promoting FV activation. PolyP also profoundly abrogates the function of tissue factor pathway inhibitor (TFPI). This appears to be secondary to accelerated FVa generation, since FXa is protected from inhibition by TFPI when incorporated into the prothrombinase complex (12). The combined effects of polyP result in an earlier peak in thrombin generation without changing the total amount of thrombin generated (11). We now report that, in the absence of AT, heparins demonstrate procoagulant activity similar of that of polyP.

Tissue factor-induced clotting in plasma immunodepleted of AT was slightly accelerated when UFH was added, but heparin markedly accelerated clot formation triggered by FXa. (Figure 1A). Maximal procoagulant activity occurred at a concentration of about 1 U/mL UFH. On the other hand, clotting time triggered by thrombin was prolonged by UFH in AT-deficient plasma (Figure 1A), presumably due to heparin-enhanced thrombin inhibition by heparin cofactor II (HCII). In plasma deficient in both AT and HCII, the addition of UFH resulted in very mild prolongation of clotting times initiated by thrombin (Figure 1B), but when clotting was initiated by FXa, addition of UFH shortened the time to clot formation (Figure 1B). These findings

Correspondence to: Stephanie A. Smith Department of Internal Medicine, College of Medicine, University of Illinois at Urbana-Champaign, 465 Medical Sciences Bldg. MC-714 506 S. Mathews, Urbana, IL 61801, USA Tel. +1 217-333-4324, Fax +1 217-265-5290 E-mail: sasmith6@uiuc.edu.

indicate that the procoagulant effect of UFH occurs upstream of thrombin and might result from accelerating the assembly of the prothrombinase complex. In the absence of both AT and HCII, maximal procoagulant activity of UFH occurred at much higher heparin concentrations (around 4 U/mL), and over a broader range of concentrations, than when HCII was available to inhibit any thrombin generated.

Heparin's ability to shorten FXa clotting times was unchanged by inhibiting the contact pathway with corn trypsin inhibitor (Figure 1C), and adding UFH to AT-deficient plasma did not shorten the plasma recalcification clotting time (data not shown). The procoagulant effect of heparin is therefore not due to contact activation.

The low molecular weight heparin, enoxaparin, exhibited a procoagulant activity similar to that observed with UFH in AT-deficient plasma (Figure 1D), indicating that shorter proteoglycans can still exert this procoagulant effect. Neither UFH nor enoxaparin resulted in as profound a shortening of clot time as did polyP (Figure 1D). TFPI anticoagulant function was abrogated by UFH over a broad range of heparin concentrations (0.05 U/mL to 1 U/mL), although the abrogation was not as profound as that observed for polyP (Figure 1E).

The procoagulant effect of UFH was no longer observed when pre-formed factor Va was added to AT-deficient plasma (Figure 1F). These findings are consistent with the idea that the procoagulant activity of heparin, like that of other negatively charged polymers (polyP and NASP), results from accelerating FV activation.

Since similar procoagulant effects have been observed with a variety of anionic polymers, the procoagulant activity of heparin is presumably charge-mediated. The specific saccharide sequence necessary for AT binding is likely not necessary for this procoagulant effect, since similarly charged NASP and polyP are procoagulant but do not catalyze protease inhibition by AT (8,11).

Sulfated proteoglycans are extremely heterogeneous in sequence, size and sulfation (13). While the net effect of pharmaceutical heparin preparations is strongly anticoagulant, our findings indicate that individual molecules within these heterogeneous preparations may also have procoagulant activity. Cell-surface HSPGs containing the AT-binding pentasaccharide play an important role in local inhibition of coagulation serine proteases, but it is tempting to speculate that HSPGs lacking the AT-binding saccharide sequence may actually have procoagulant properties (by serving to accelerate FV activation). The spectrum of HSPGs expressed by endothelial cells may therefore represent a potential therapeutic target for regulation of coagulation in disease states.

The specific structural requirements for the procoagulant effect of heparin remain to be elucidated. Identification of the specific size, saccharide sequence, and/or charge requirements may allow for rational pharmaceutical design of proteoglycans with specific anticoagulant or procoagulant properties.

References

1. Tollefsen, DM.; Zhang, L. Hemostasis and Thrombosis. 5th ed.. 2006. Lippincott Williams and Wilkins; Heparin and Vascular Proteoglycans; p. 271-283.
2. Fonseca RJ, Mourao PA. Fucosylated chondroitin sulfate as a new oral antithrombotic agent. *Thromb Haemost* 2006;96:822–829. [PubMed: 17139379]
3. Melo FR, Mourao PA. An algal sulfated galactan has an unusual dual effect on venous thrombosis due to activation of factor XII and inhibition of the coagulation proteases. *Thromb Haemost* 2008;99:531–538. [PubMed: 18327401]

4. Lindahl U. Heparan sulfate-protein interactions--a concept for drug design? *Thromb Haemost* 2007;98:109–115. [PubMed: 17598000]
5. Marcum JA, Atha DH, Fritze LM, et al. Cloned bovine aortic endothelial cells synthesize anticoagulant active heparan sulfate proteoglycan. *J Biol Chem* 1986;261:7507–7517. [PubMed: 2940242]
6. Kojima T, Leone CW, Marchildon GA, et al. Isolation and characterization of heparan sulfate proteoglycans produced by cloned rat microvascular endothelial cells. *J Biol Chem* 1992;267:4859–4869. [PubMed: 1537864]
7. Young E. The anti-inflammatory effects of heparin and related compounds. *Thromb Res*. In press
8. Liu T, Scallan CD, Broze GJ Jr. et al. Improved coagulation in bleeding disorders by Non-Anticoagulant Sulfated Polysaccharides (NASP). *Thromb Haemost* 2006;95:68–76. [PubMed: 16543964]
9. Mutch NJ, Johnson KW, Morrissey JH. Non-anticoagulant sulfated polysaccharides enhance thrombin generation by accelerating factor V activation. *J Thromb Haemost* 2007;5(S2):O-M-094
10. Kornberg A, Rao NN, Ault-Riche D. Inorganic polyphosphate: a molecule of many functions. *Annu Rev Biochem* 1999;68:89–125. [PubMed: 10872445]
11. Smith SA, Mutch NJ, Baskar D, et al. Polyphosphate modulates blood coagulation and fibrinolysis. *Proc Natl Acad Sci U S A* 2006;103:903–908. [PubMed: 16410357]
12. Mast AE, Broze GJ Jr. Physiological concentrations of tissue factor pathway inhibitor do not inhibit prothrombinase. *Blood* 1996;87:1845–1850. [PubMed: 8634431]
13. Fareed J, Hoppensteadt D, Schultz C, et al. Biochemical and pharmacologic heterogeneity in low molecular weight heparins. Impact on the therapeutic profile. *Curr Pharm Des* 2004;10:983–999. [PubMed: 15078128]

Figure 1. Procoagulant activity of heparins in AT-deficient plasma

A) UFH was added to AT-deficient plasma (from Affinity Biologicals), after which clotting was initiated by either FXa (●), tissue factor (■) or thrombin (▲). B) UFH was added to plasma deficient in both AT and HCII, after which clotting was initiated by FXa (●) or thrombin (▲). C) UFH was added to AT-deficient plasma with (▽) or without (●) 250 µg/ml corn trypsin inhibitor, after which clotting was initiated by FXa. D) UFH (●), enoxaparin (○), or polyP (△) were added to AT-deficient plasma, after which clotting was initiated by FXa. E) UFH (●) or polyP (△) were added to AT-deficient plasma spiked with 400 ng/mL TFPI, after which clotting was initiated by FXa. The dashed line indicates clotting time without added TFPI. F) UFH was added to AT-deficient plasma with (□) or without (●) 1 nM added FVa, after which clotting was initiated by FXa.

Clotting was quantified at 37°C in a STart 4 coagulometer in reactions containing 50 µl plasma and 100 µl reagent (prepared in 25 mM Tris HCl pH 7.4, 50 mM NaCl, 0.05% BSA, 12.5 mM CaCl₂ and 0.01% NaN₃). FXa reagent contained 75 pM human FXa and 50 µM phospholipid (20% phosphatidylserine, 80% phosphatidylcholine; vesicles made by sonication). Tissue factor reagent contained 120 pM relipidated recombinant human tissue factor and 50 µM phospholipid. Thrombin reagent contained 2.8 nM human α thrombin.