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Characterization of the plasma and blood anticoagulant potential of structurally and mechanistically novel oligomers of 4-hydroxycinnamic acids

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Abstract

Recently, we designed sulfated dehydropolymers (DHPs) of 4-hydroxycinnamic acids that displayed interesting anticoagulant properties. Structurally and mechanistically, sulfated DHPs are radically different from all the anticoagulants studied to date. To assess whether their unique mechanism and structure is worth exploiting for further rational design of homogeneous DHP-based molecules, we investigated their anticoagulant potential in human plasma and blood using a range of clotting assays. Sulfated DHPs prolong plasma clotting times, prothrombin and activated partial thromboplastin times at concentrations comparable to the clinically used low-molecular-weight heparin, enoxaparin. Fibrin formation studies on human plasma show that there is a structural dependence of anticoagulant action. Human whole blood studies using thromboelastography and hemostasis analysis system indicate that they are 17–140-fold less potent than enoxaparin. Results demonstrate that sulfated DHPs possess good in-vitro and ex-vivo activity, which will likely be improved through a rational design.

Keywords

anticoagulant agents; clotting times; dehydropolymers; hemostasis analysis system; thrombin inhibition; thromboelastography

Introduction

Thrombin and factor Xa (FXa), two key serine proteases of the coagulation cascade, have been the prime target of rational drug design for the last decade [1]. Both proteases can be targeted through either the antithrombin (AT)-dependent (indirect) or AT-independent (direct) inhibition pathways. Direct inhibitors include peptides or peptidomimetics, for example, hirudin, argatroban and ximelagatran, whereas indirect inhibitors include heparin, low-molecular-weight heparins (LMWHs) and fondaparinux. Heparins work through AT, a plasma

serine proteinase inhibitor (serpin) and a major natural regulator of clotting. Full-length heparin, or unfractionated heparin (UFH) (Fig. 1a), greatly enhances the rate of AT inhibition of thrombin, FXa and factor IXa under physiological conditions, which is the major mechanism involved in its anticoagulant action [2]. Yet, UFH suffers from several limitations, including bleeding risk, variable patient response, heparin-induced thrombocytopenia and the inability to inhibit clot-bound thrombin [3,4]. LMWHs, derivatives of UFH with reduced polymeric length, and fondaparinux, a specific sequence of five saccharide residues (Fig. 1a), have been introduced in the past two decades as agents with higher specificity for FXa. Another heparin pentasaccharide, idraparinux, is likely to be introduced shortly [5,6]. Yet, each newer agent retains bleeding risk and is unable to inhibit clot-bound thrombin [7,8].

The problems of heparin-based therapy arguably arise from the structure of UFH. The large number of sulfate groups introduces phenomenal anionic character in the polysaccharide. The average molecular weight of UFH is approximately 15 000, implying the presence of approximately 65–85 negative charges on average on a single chain [9]. In addition to this polyanionic character, heparin biosynthesis results in millions of sequences that differ from each other in the placement of sulfate groups, thereby generating considerable microheterogeneity and polydispersity. Both these structural features introduce a large number of interactions with plasma proteins and cells [10], which are potentially the cause of heparin's adverse effects.

With respect to the other pathway of regulating thrombin and FXa, the direct inhibition pathway, several molecules such as argatroban, ximelagatran and dabigatran for thrombin and rivaroxaban, DX9065a and razaxaban for FXa have been put forward [11–13]. Direct thrombin inhibitors and FXa inhibitors form a major class of clotting regulators that are considered to be superior to heparins primarily because of the expectation that they can inhibit both circulating and clot-bound enzymes. Yet, challenges exist in the development of these inhibitors, including establishing enzyme-binding affinity that is not associated with excessive bleeding and avoiding liver toxicity [14].

We reasoned that reducing UFH's high negative charge density would reduce its adverse effects. At the same time, enhancing its hydrophobic character would possibly induce greater specificity of action. Thus, we designed sulfated dehydropolymers (DHPs) of 4-hydroxycinnamic acids as advanced mimics of UFH and LMWH (Fig. 1b). Sulfated DHPs are typically prepared in high yields in two simple steps, an enzymatic coupling of 4-hydroxycinnamic acid monomers followed by a chemical sulfation step [15]. Initial studies suggested that the designed sulfated DHPs could reduce clotting [15]. Recently, we discovered that although designed as mimics of heparin, sulfated DHPs do not prefer to utilize the indirect pathway of thrombin and FXa inhibition. In fact, the dominant mechanism of inhibition is direct allosteric inhibition of thrombin and FXa [16]. Allosteric inactivation of the two key proteinases arises from binding in anion-binding exosite II. This is the first observation of thrombin inhibition arising from exclusive exosite II interaction. The dominant mechanism of FXa inhibition remains unstudied but is likely to involve its anion-binding exosite II.

In addition to this unique mechanistic feature, sulfated DHPs are also structurally distinct. Except for the presence of sulfate groups, DHPs possess a scaffold unlike any other anticoagulant investigated to date. The unique mechanism of action and novel structure of DHPs may lead to a homogeneous molecule that exhibits more specificity and reduced side effects in comparison with current agents. However, before a homogeneous molecule can be designed, it is important to answer the question whether DHPs possess sufficient anticoagulant activity in plasma and blood for further rational design. To answer this question, we studied the anticoagulant potential of DHPs in several in-vitro and ex-vivo systems, including activated partial thromboplastin time (APTT), prothrombin time (PT), thromboelastography (TEG) and

hemostasis analysis system (HAS), and compared it with a clinically used anticoagulant, enoxaparin. Our studies show that sulfated DHPs are fairly potent anticoagulants in human plasma and blood. These results support the need to explore the DHP scaffold in the design of novel anticoagulants.

Methods

Proteins, chemicals and coagulation assay conditions

Sulfated DHPs, CDSO₃, FDSO₃ and SDSO₃ (Fig. 1b), were prepared in two steps from 4-hydroxycinnamic acid monomers, caffeic acid, ferulic acid and sinapic acid, as described previously [15]. Stock solutions of sulfated DHPs were prepared in deionized water and stored at -80°C. Pooled normal human plasma for coagulation time assays was purchased from Valley Biomedical (Winchester, Virginia, USA). APTT reagent-containing ellagic acid (APTT-LS), thromboplastin-D and 25 mmol/l CaCl₂ were obtained from Fisher Diagnostics (Middletown, Virginia, USA). Thromboelastograph Coagulation Analyzer 5000 (TEG), disposable cups and pins and 200 mmol/l stock CaCl₂ were obtained from Haemoscope Corporation (Niles, Illinois, USA). LMWH (molecular weight 5060) was purchased from Sigma (St. Louis, Missouri, USA), whereas enoxaparin (molecular weight 4500) was from Aventis Pharmaceuticals (Bridgewater, New Jersey, USA). All other chemicals were analytical reagent grade from either Sigma Chemicals (St. Louis, Missouri, USA) or Fisher (Pittsburgh, Pennsylvania, USA) and used as obtained.

Inhibition of CaCl₂-initiated fibrin formation in plasma by sulfated dehydropolymers

A 650 µl aliquot of freshly thawed pooled human plasma was coincubated with 10 µl sulfated DHP (either 3 or 5 mg/ml in H₂O) and 200 µl APTT-LS reagent at 37°C for 5 min. Following incubation, 850 µl of this sample was transferred to a PEG 20 000-coated polystyrene cuvette. Fibrin formation was initiated by rapidly adding 50 µl of 20 mmol/l Tris-HCl buffer, pH 7.4, containing 100 mmol/l NaCl, 25 mmol/l CaCl₂ and 0.1% PEG8000, and 200 µl of 25 mmol/l CaCl₂. Following initiation, the transmittance at 600 nm was continuously monitored until a plateau was reached corresponding to the formation of a solid fibrin polymer.

Inhibition of thromboplastin-D-initiated fibrin formation in plasma by sulfated dehydropolymers

A 200 µl aliquot of freshly thawed citrated human plasma was coincubated with 10 µl sulfated DHP (or the reference molecule) and 390 µl of H₂O at 37°C for 5 min. Following incubation, 600 µl of the sample was transferred to a PEG 20 000-coated polystyrene cuvette. Clotting was initiated by rapidly adding 400 µl of prewarmed thromboplastin-D reagent, and the decrease in transmittance at 600 nm was monitored continuously until a plateau was reached. The time to clot, or the lag time, was calculated as the time necessary for the transmittance to decrease by 1% from the initial value. Likewise, the time necessary for a 50% decrease in transmittance from the initial value was also obtained.

Prothrombin time and activated partial thromboplastin time

Clotting time (CT) was determined in a standard 1-stage recalcification assay with a BBL Fibrosystem fibrometer (Becton-Dickinson, Sparks, Maryland, USA). For PT and APTT assays, the reagents were prewarmed to 37°C. For PT assays, 10 µl sulfated DHP (or the reference molecule) was mixed with 90 µl of citrated human plasma, incubated for 30 s at 37°C followed by addition of 200 µl prewarmed thromboplastin. For APTT assays, 10 µl sulfated DHP was mixed with 90 µl citrated human plasma and 100 µl 0.2% ellagic acid. After incubation for 220 s, clotting was initiated by adding 100 µl of 25 mmol/l CaCl₂. Each experiment was performed at least twice. The averaged data was fitted by a quadratic equation

to calculate the concentration of the anticoagulant necessary to double the CT ($2 \times$ APTT or $2 \times$ PT).

Thromboelastograph analysis of clot formation in the presence of sulfated dehydropolymers

TEG assays were performed essentially as reported earlier [17]. Briefly, the assays were initiated by transferring 20 μ l of 200 mmol/l CaCl_2 into the hemoscope disposable cup, oscillating through $4^\circ 45'$ angle at 0.1 Hz, followed by the addition of a mixture of 340 μ l of sodium citrated whole blood containing 10 μ l sulfated DHP or dH_2O (control) at 37°C . This recalcification initiates clot formation in the TEG coagulation analyzer, which operates until all necessary data collection [reaction time (R), K , angle α and maximum amplitude] is completed in an automated manner.

Hemostasis analysis system analysis of clot formation in the presence of sulfated dehydropolymers

Analysis of platelet function and clot structure was performed using the HAS (Hemodyne, Inc., Richmond, Virginia, USA). A mixture of 700 μ l of citrated whole blood and 10 μ l sulfated DHP or ddH_2O (control) was coincubated at room temperature for 5 min, and then, 700 μ l was placed in a disposable cup. To initiate clotting, 50 μ l of 150 mmol/l CaCl_2 was added to 700 μ l of the blood–DHP mixture to give a final CaCl_2 concentration of 10 mmol/l, while the cone was simultaneously lowered into the recalcified blood sample. As the clotting proceeds, platelets attach to both surfaces, generating tension within the fibrin meshwork. This tension is measured with a displacement transducer in terms of platelet contractile force (PCF). The onset of PCF is a measure of thrombin generation time (TGT), whereas clot elastic modulus (CEM) is the ratio of the applied force (stress) by the transducer to the measured displacement (strain). The HAS system operates in an automated manner until all data is collected.

Results and discussion

Structure of sulfated dehydropolymers of 4-hydroxycinnamic acids

Three synthetic sulfated DHPs – CDSO_3 , FDSO_3 and SDSO_3 (Fig. 1b) – were studied. The molecules were prepared in two steps from caffeic acid, ferulic acid and sinapic acid, each of which contains a common scaffold, the 4-hydroxycinnamic acid monomer (Fig. 1b) [15]. Briefly, sulfated DHPs (molecular weight ~ 2500 – 4000) are a mixture of oligomeric chains that contain 4–15 monomers, suggesting that the molecules are comparable in size to enoxaparin (molecular weight ~ 5000) [15]. In addition, DHPs contain several types of intermonomeric linkages (Fig. 1b), thereby generating polydispersity and heterogeneity, a property they share with LMWHs. Yet, sulfated DHPs are significantly less sulfated than heparins. Whereas sulfated DHPs contain an average of 0.33 sulfate group per monomer, LMWHs possess an average of 1–1.3 sulfate groups for every saccharide residue. More importantly, sulfated DHPs possess a large number of aromatic rings in the backbone, whereas heparins have none. Thus, sulfated DHPs are significantly more hydrophobic than LMWHs.

Effect of sulfated dehydropolymers on fibrin formation in normal human plasma

To determine whether our sulfated DHPs prolong fibrin formation in plasma, we utilized in-vitro transmittance assays. Addition of CaCl_2 to normal pooled human plasma under APTT-like conditions triggers ‘coagulation’, resulting in the synthesis of fibrin, which blocks the passage of light through the sample. A characteristic decrease in transmittance at 600 nm as a function of time is observed from which the time to clot and the time it takes to reduce the transmittance, that is, clotting, by 50% (T_{50}) can be measured. The presence of all three sulfated DHPs prolonged fibrin synthesis, as shown by the delayed decrease in transmittance at 600 nm (Fig. 2a). For SDSO_3 , the T_{50} value changed from 79 to 161 s as the concentration was increased

from 27 to 45 $\mu\text{g/ml}$ (Fig. 2a, Table 1). Similarly, T_{50} values were 272 and 593 s and 93 and 247 s at 27 and 45 $\mu\text{g/ml}$ for CDSO_3 and FDSO_3 , respectively. These initial results demonstrated that sulfated DHPs prolong fibrin formation in a dose-dependent manner. The results also suggest that the anticoagulation potency varied with the structure of the sulfated DHP (Fig. 1).

To assess whether the anticoagulant potency of sulfated DHPs is retained if coagulation is initiated through the extrinsic pathway, thromboplastin-D was used as an initiator of clotting. Once again, the presence of all three sulfated DHPs significantly slowed down the formation of fibrin in normal plasma, suggesting that three molecules inhibit clotting (not shown). Figure 2b and c show the change in time to clot and T_{50} as a function of the concentration of each sulfated DHP (and reference molecule, enoxaparin). Both time to clot and T_{50} increase as the concentration of sulfated DHP increases. The increase is not linear and is accelerated at higher concentrations of the anticoagulant. More interestingly, the three sulfated DHPs display an anticoagulation profile similar to enoxaparin. Whereas CDSO_3 is more potent than enoxaparin, FDSO_3 and SDSO_3 are less potent. For example, the concentration of anticoagulant needed to double the time for 50% fibrin formation was found to be approximately 3.5 $\mu\text{mol/l}$ for CDSO_3 and 6, 11 and more than 25 $\mu\text{mol/l}$ for enoxaparin, FDSO_3 and SDSO_3 , respectively.

Effect of sulfated dehydropolymers on clotting times

PT and APTT are commonly used to assess the coagulation status of human plasma [18]. All three sulfated DHPs exhibited a significant concentration-dependent prolongation of PT and APTT (not shown). A typical parameter for describing anticoagulant activity in these assays is the concentration of the anticoagulant needed for doubling the normal plasma CT ($2 \times \text{PT}$ or $2 \times \text{APTT}$). The $2 \times \text{PT}$ value for sulfated DHPs ranged from 13.1 to 33.3 $\mu\text{mol/l}$, whereas that for enoxaparin was 75.3 $\mu\text{mol/l}$, suggesting that the new molecules are 2.3–5.7-fold more potent in the PT assay (Table 1). The doubling of APTT required 2.9–6.4 $\mu\text{mol/l}$ concentration of the three sulfated DHPs, whereas enoxaparin required 1.2 $\mu\text{mol/l}$. This indicates that sulfated DHPs are approximately 2.4–5.3-fold weaker anticoagulants in the APTT assay as compared with enoxaparin, and the order of activity is $\text{CDSO}_3 > \text{FDSO}_3 > \text{SDSO}_3$ (Table 1).

Thromboelastographic measurement of the effect of sulfated dehydropolymers on whole blood clotting

Whole blood clotting is a dynamic process that involves many components, including cells, which may alter anticoagulant potency. To compare sulfated DHPs and enoxaparin in a whole blood system, we employed TEG, a technique used in clinical settings for following anticoagulation with LMWHs [19–21]. TEG measures various responses of a formed clot to shearing force. In this technique, a pin is inserted into an oscillating cup containing whole blood. As fibrin polymerizes, the pin starts to move with the oscillating cup, and the movement of the pin is recorded as amplitude, which in time reaches maximum amplitude (Fig. 3a). The stronger the clot, the more the pin moves with the cup and the higher the maximum amplitude. Shear elastic modulus strength (G), a measure of clot stiffness, is calculated from maximum amplitude. Additionally, R and angle α (Fig. 3a) are also obtained in a TEG experiment. R is the time required for the initial fibrin formation, whereas α is the acute angle in degrees between an extension of the R tracing and the tangent of the maximum slope produced by the TEG tracing during clot stiffening. Angle α is a measure of the rate of formation of three-dimensional fibrin network. Parameters that affect maximum amplitude include fibrin concentration and structure, concentration and functional state of platelets, deficiency of coagulation factors and the presence of clotting inhibitors [22].

All three sulfated DHPs affect R , α , maximum amplitude and G parameters in a dose-dependent manner (Table S1 in Supplementary Material). Briefly, as the concentration of CDSO_3

increases from 0 to 24.3 $\mu\text{mol/l}$, R increases from 7.0 to 21.5 min. This effect parallels the time to clot results obtained in the plasma assay. Likewise, sulfated DHPs lower the value of angle α from 59° for normal blood to 13.5–17° at the highest concentrations studied. This indicates that the kinetics of fibrin polymerization and networking is significantly retarded by the presence of sulfated DHPs. Enoxaparin exhibits similar characteristics, except that it is 23–51-fold more potent than sulfated DHPs when comparisons are made at doubling the R value from its value in the absence of any anticoagulants (not shown). Likewise, enoxaparin is 17–32-fold and 18–37-fold more potent when comparisons are made for a 50% reduction in the angle α and shear elastic modulus G , respectively (Fig. 3b).

Effect of sulfated dehydropolymers on whole blood coagulation as evaluated by hemostasis analysis system

To further compare the whole blood anticoagulant potential of sulfated DHPs with enoxaparin, we performed an ex-vivo study using HAS, which measures the forces generated by platelets within a clot [23]. In this technique, the clot is allowed to form between a temperature-controlled lower surface (cup) and a parallel upper surface (cone). As the clot grows, it attaches to both the surfaces, pulling the fibrin strands inward. This pull is measured by a displacement transducer, which produces an electrical signal on the cone proportional to the amount of force generated by the platelets. HAS also provides detailed information on clot structure through the measurement of CEM, which is the ratio of stress induced by platelets to strain arising from the change in clot thickness [24]. PCF is observed to increase as soon as thrombin is formed, suggesting that the appearance of PCF can be used as surrogate marker for TGT, the minimal time required for production of thrombin following initiation of clotting [23].

In addition to its dependence on thrombin, PCF is sensitive to platelet number, platelet metabolic status, presence of thrombin inhibitors and the degree of glycoprotein IIb/IIIa exposure [20,25–27]. Likewise, CEM is a complex parameter that is sensitive to changes in clot structure, fibrinogen concentration, the rate of thrombin generation and red blood cell flexibility, whereas TGT is sensitive to clotting factor deficiencies, antithrombin concentration and the presence of anticoagulants. Low PCF and CEM coupled with a prolonged TGT are associated with increased bleeding risk, whereas elevated PCF and CEM paired with a decreased TGT are associated with thrombotic disease states.

All three DHPs affect TGT, PCF and CEM parameters in a dose-dependent manner (Table S2 in Supplementary Material). For example, as the concentration of FDSO₃ increases from 0 to 23.8 $\mu\text{mol/l}$, the TGT value increases from 235 to 465 s (Fig. 4a). This effect parallels the results obtained in the plasma thrombogenesis assay and TEG. More importantly, the presence of sulfated DHPs in blood decreases PCF from 7.6 to 2.4–1.2 kdynes at 14–37 $\mu\text{mol/l}$ (Fig. 4b), whereas enoxaparin induces a PCF of 0.9 kdynes at 0.44 $\mu\text{mol/l}$. When comparisons are made for a 50% reduction in PCF, enoxaparin is 63–140-fold more potent. Likewise, sulfated DHPs decrease CEM from 21.6 kdynes/cm² for normal blood to 4.5–1.3 kdynes/cm² at the highest concentrations studied. Comparison of CEM values indicates that enoxaparin is 43–90-fold more potent than sulfated DHPs (Fig. 4c). These results confirm that sulfated DHPs behave in a manner similar to enoxaparin, except for the concentration at which these are effective.

Conclusion

The major conclusion of this work is that sulfated DHPs display whole blood anticoagulation properties similar to a clinically used anticoagulant, enoxaparin, although the effective concentration range is different. In TEG and HAS assays, sulfated DHPs are 17–51-fold and 43–140-fold less active than enoxaparin, respectively. Considering that the structure and mechanism of action of sulfated DHPs is radically different from all known anticoagulants

[15,16], this is a significant observation. It implies that a new class of more potent molecules, most likely homogeneous and synthetically accessible, may be possible to design from the DHP scaffold. We expect that this class of molecules will utilize allosteric modulation of thrombin and FXa activity through binding in exosite II, the first molecules to display this unique mechanism [16]. In addition, our previous results using enzyme inhibition assays show that unsulfated DHPs also possess significant anticoagulation potential [15]. This suggests that it may be possible to design unsulfated, synthetic molecules with a unique mechanism of action. A specific advantage expected of these unsulfated homogeneous DHP-based structures is that the absence of sulfate group would make the molecules orally bioavailable.

The work presented here shows that in the PT assay, the three sulfated DHPs are effective at concentrations in the range of enoxaparin, whereas in the APTT assay, they are only 2–6-fold weaker. Despite major mechanistic differences, both sulfated DHPs and enoxaparin prolong APTT better than PT. Inhibition of fibrin formation in plasma shows that CDSO₃ was comparable to enoxaparin. Yet, sulfated DHPs are much weaker in whole blood than enoxaparin. It is possible that the significant hydrophobic character of sulfated DHPs induces binding to cells, resulting in significant sequestering of active agent. It is likely that this nonspecific binding will be reduced with homogeneous, synthetic small molecules.

Overall, the results demonstrate that sulfated DHPs possess good plasma and whole blood anticoagulation activity. This does not imply that our novel molecules will be clinically effective. Toxicity studies will have to be performed to ascertain that these novel structures do not induce abnormal effects. An important point to note in this regard is that in-vivo enoxaparin does not prolong PT and APPT at concentrations sufficient to anticoagulate, suggesting that in-vitro or ex-vivo potency does not translate directly into in-vivo effectiveness. Yet, the results described here suggest that the novel structure and mechanism of sulfated DHPs may lead to a new class of potent anticoagulants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Structures of heparins (a) and sulfated dehydropolymers (b). (a) Fondaparinux is based on heparin pentasaccharide, whereas heparin and LMWHs are polydisperse, heterogeneous mixture of polysaccharide chains (molecular weight $\sim 15\,000$ and ~ 5000 Da, respectively) arising because of variations in X, Y, Z and R groups. (b) Sulfated DHPs possess a radically different structure from the heparins (and other anticoagulants) and are synthesized in two steps from the corresponding 4-hydroxycinnamic acid monomers, CA, FA or SA. The molecular weight of sulfated DHPs is in the range of 2500–4000. Linkages, β -O-4 and β -5, are commonly present in sulfated DHPs (shown as shaded ovals). CA, caffeic acid; DHPs, dehydropolymers; FA, ferulic acid; HRP, ; LMWHs, low-molecular-weight heparins; SA, sinapic acid.

Fig. 2.

Inhibition of fibrin formation in pooled normal human plasma in the presence of sulfated dehydropolymers. Fibrin formation as a function of time was monitored using the decrease in transmittance of light at 600 nm following initiation of 'clotting' under APTT conditions (a) and PT conditions (b). (a) CDSO₃ (bold black lines), FDSO₃ (bold gray lines) and SDSO₃ (thin black lines). Concentrations of sulfated DHPs used were either 27 µg/ml (denoted as 'L') or 45 µg/ml ('H'). (b) TC and (c) T₅₀ values are plotted as a function of the concentration of sulfated DHP and enoxaparin. Solid lines in both (b) and (c) are trend lines, not nonlinear regressions. APTT, activated partial thromboplastin time; DHPs, dehydropolymers; PT, prothrombin time; T₅₀, time needed for 50% formation of fibrin; TC, time to clot; Δ, CDSO₃; (◆), enoxaparin; (●), FDSO₃; (□), SDSO₃ [see text for details].

Fig. 3.

Comparison of the effect of sulfated dehydropolymers and enoxaparin on clot formation in whole blood using TEG. Inset in (a) shows a typical thromboelastogram expected of any anticoagulant. MA, R , α and G are parameters obtained from TEG analysis [see Methods for details]. (b) Shows the variation in G as a function of concentration of the sulfated DHPs and enoxaparin. Solid lines are trend lines (not regression fits) from which concentration of anticoagulant needed to reduce shear elastic modulus G by 50% (shown as shaded line) of the starting value was derived. DHPs, dehydropolymers; MA, maximum amplitude; TEG, thromboelastography.

Fig. 4.

Comparison of the effect of sulfated dehydropolymers and enoxaparin on platelet function in whole blood using hemostasis analysis system. (a) Shows selected HAS profiles obtained with FDSO₃; (b) and (c) show the variation in PCF and CEM, respectively, as a function of concentration of the sulfated DHPs and enoxaparin. Solid lines are trend lines from which the concentration of anticoagulant needed to reduce PCF or CEM by 50% (shaded line) of the starting value was derived. CEM, clot elastic modulus; DHPs, dehydropolymers; HAS, hemostasis analysis system; PCF, platelet contractile force; TGT, thrombin generation time.

Table 1
Effect of sulfated dehydropolymers and enoxaparin on human plasma clotting using fibrin formation and clotting time assays

	Fibrin formation assay		Clotting time assay	
	27 $\mu\text{g/ml}$ (s)	45 $\mu\text{g/ml}$ (s)	$2 \times \text{PT}^a$ ($\mu\text{mol/l}$)	$2 \times \text{APTT}^b$ ($\mu\text{mol/l}$)
CDSO ₃	272	593	13.1 ± 1.3	2.9 ± 0.8
FDSO ₃	93	247	16.6 ± 1.7	4.1 ± 0.4
SDSO ₃	79	161	33.3 ± 3.0	6.4 ± 1.2
Enoxaparin	_c	–	75.3 ± 2.0	1.2 ± 1.1

APTT, activated partial thromboplastin time; PT, prothrombin time.

^aThe normal human plasma PT was found to be 12.2 s.

^bThe uninhibited human plasma APTT was 29.5 s.

^cNot performed.