

The effects of unfractionated heparin, low molecular weight heparin and danaparoid on the thromboelastogram (TEG): an in-vitro comparison of standard and heparinase-modified TEGs with conventional coagulation assays

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To investigate the effects of unfractionated heparin (UFH), low molecular weight heparin (LMWH) and danaparoid (DPD) added to whole blood *in vitro* on standard and heparinase-modified thromboelastogram (TEG) parameters compared with conventional assays of coagulation. The effects of UFH, LMWH and DPD on standard TEG parameters were compared with the prothrombin time, activated partial thromboplastin time, thrombin time and anti-activated factor X (anti-FXa) activity, at concentrations of these anticoagulants ranging from 0.025 to 1 U/ml. In the second part of the study, the effects of very low concentrations (0.005–0.05 U/ml) of UFH, LMWH and DPD on the difference between standard and heparinase-modified TEG parameters were compared with the prothrombin time, activated partial thromboplastin time, thrombin time and anti-FXa activity. Standard TEG parameters were outside the reference range at lower concentrations of UFH, LMWH and DPD than most conventional coagulation assays were able to detect. Only anti-FXa activity was more sensitive to the presence of these anticoagulants than the standard TEG alone. The lowest concentration of UFH, LMWH and DPD used in this study (0.005 U/ml) caused significant differences between the standard and heparinase-modified α -angles of the TEG. In addition, the difference between standard and

heparinase-modified TEG parameters distinguished between low concentrations (0.005–0.05 U/ml) of UFH with greater sensitivity than anti-FXa activity, but were less sensitive to LMWH and DPD. The standard TEG is more sensitive to UFH, LMWH and DPD than most conventional coagulation tests, with the exception of anti-FXa activity. Calculation of the difference between standard and heparinase-modified TEG parameters greatly increases the sensitivity of the assay for the effects of these anticoagulants, and is more sensitive to very low quantities of UFH than anti-FXa activity. *Blood Coagul Fibrinolysis* 17:97–104 © 2006 Lippincott Williams & Wilkins.

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Introduction

Thromboelastography was first developed in 1948 [1] and has been used extensively as a research tool, but only more recently has it been employed during liver transplantation and cardiac surgery to assess coagulation and guide blood product replacement in the peri-operative period [2–4]. The unpopularity of the thromboelastogram (TEG) as a coagulation test was partly due to poor understanding of how the TEG is affected by abnormalities in the coagulation system, how these variables correlate with conventional clotting tests and how the investigation should be standardized [5–7]. In the past few years, a number of studies re-investigating the properties of the TEG have been published, prompted by the increasing use of the TEG in clinical practice [8–14].

Thromboelastography provides a dynamic and global assessment of coagulation that is not addressed by most conventional laboratory coagulation tests (a full review of TEG methodology can be found in reference [15]). Conventional assays terminate with the formation of a fibrin clot, and therefore possess a well-defined end point that is easily standardized. Segments of the clotting cascade are artificially isolated in these assays, and therefore they do not reflect other haematological abnormalities that may interfere with the formation of a stable fibrin clot, such as impaired platelet function. In contrast, the TEG begins to generate data at the point at which fibrin strands begin to form, and as such is a more global and dynamic investigation, with several measurable endpoints [5]. It reflects the interaction between platelets, the clotting cascade and fibrinolysis. The TEG is

sensitive to qualitative and quantitative differences in platelets, proteins of the clotting cascade and fibrinolysis [7,15].

The TEG trace can be modified by a number of factors, but because of the increasing use of the TEG during surgical procedures in which the administration of heparin is routine, the effects of heparin and related compounds have been the most extensively studied [9,12,16–19]. Commercially available TEG cuvettes coated with heparinase I can be used to inactivate heparin and other glycosaminoglycans (GAGs), such as heparan sulphate, for the assessment of the TEG in patients who have received heparin [3,20–22]. However, little has been published regarding the correlation between the variables of the standard and heparinase-modified TEG with conventional clotting tests. The present in-vitro study was divided into two parts. In the first part, the effects of unfractionated heparin (UFH), low molecular weight heparin (LMWH) and danaparoid (DPD) on standard TEG parameters was compared with the prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT) and anti-activated factor X (anti-FXa) activity over the concentration range 0.025–1 U/ml. In the second part, the difference between standard and heparinase-modified TEG parameters was compared with the PT, aPTT, TT and anti-FXa activity at very low concentrations of UFH, LMWH and DPD (0.005–0.05 U/ml).

Materials and methods

Heparins and danaparoid

UFH was supplied by CP Pharmaceuticals Ltd (Wrexham, UK). LMWH [Dalteparin (Fragmin)] was supplied by Pharmacia & Upjohn (Milton Keynes, UK). Danaparoid (Orgaran) was supplied by B & S Durbin Ltd (Harrow, Middlesex, UK).

Participants

This study was performed as part of a larger ongoing study of thromboelastography in various medical disorders, which has been approved by the local research ethics committee of the Royal Free Hospital. Blood samples were taken from 10 healthy male volunteers for each part of the study. For all subjects, the baseline haemoglobin, white cell count, platelet count, PT, aPTT, TT, anti-FXa activity, standard TEG and heparinase I-modified TEG were all within the reference ranges for these investigations.

Blood sampling

Blood samples were collected into plastic 10 ml Sarstedt Monovette (51582, Nüembrecht, Germany) vacuum tubes containing sodium citrate solution (final concentration, 0.106 mol/l), using a 19 G needle and no tourniquet. The study was divided into two overlapping parts in order to allow a wide range of anticoagulant

concentrations to be assessed, while avoiding storage of citrated blood samples for excessive periods of time before TEG analysis could be performed. In both parts of the study, blood was taken from each subject on one occasion, except for three subjects in the first part of the study used as controls, who were bled on three separate occasions (once per anticoagulant tested). This was necessary in order to maintain the same temporal sequence of sample testing throughout the study, because analysis of saline control samples in parallel with anticoagulant-spiked samples reduced the total number of TEG channels available at each concentration tested.

In the first part of the study, a 10 U/ml solution of LMWH, UFH or DPD was diluted 1/10 with citrated whole blood, and serial dilutions were made using citrated blood to achieve final concentrations of 1.0, 0.5, 0.25, 0.1, 0.05 and 0.025 U/ml. In the second part of the study, serial dilutions of LMWH, UFH or DPD were made with citrated blood to final concentrations of 0.05, 0.025, 0.010 and 0.005 U/ml. After phlebotomy, all samples were allowed to stabilize in citrate for at least 1 h prior to TEG analysis [10,23].

Saline control samples were prepared for three subjects in the first part of the study in order to exclude a dilutional effect on blood coagulability from the addition of these drugs, and to determine sample stability over the time from phlebotomy to completion of all TEGs. An equal volume of 0.9% (isotonic) saline instead of UFH, LMWH or DPD was added to each sample and diluted with citrated blood using the same protocol (i.e. six saline dilutions equivalent to the 1.0, 0.5, 0.25, 0.1, 0.05 and 0.025 U/ml concentrations of each anticoagulant). Since the three control subjects were bled on three separate occasions (once for each anticoagulant), a total of nine saline controls were prepared for each of the six dilutions tested. The TEG parameters and conventional coagulation test results from these control samples demonstrated limited inter-subject variability and confirmed sample stability by producing consistent results over the time period of each experiment. In addition, control results were all within the reference ranges for each parameter, excluding a dilutional effect from the addition of anticoagulants to blood. Further saline controls were therefore not prepared for the remaining subjects, so that all three anticoagulants could be tested with blood collected on a single occasion.

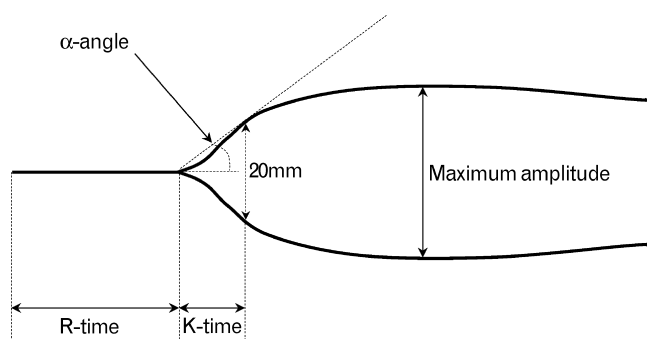
Thromboelastography

Disposable standard and heparinase I-coated plastic cuvettes and pins were supplied by Medicell UK (for Haemoscope Corp., Skokie, Illinois, USA). Two Haemoscope Thromboelastograph coagulation analysers were used for this study, with up to six TEG channels depending on availability. Twenty microlitres of 30 mmol calcium chloride were added to a 340 µl aliquot of test

blood (total volume, 360 μ l) in the cuvette to reverse citrate chelation, and were mixed by raising and lowering the TEG pin three times. A thin film of mineral oil was floated on the surface of the blood to prevent evaporation during analysis. The cuvettes were pre-heated to 37°C in the analyser.

The same temporal sequence was adopted for all samples, with the lowest concentrations of each drug analysed first and the highest concentrations last, at 1-h intervals starting 1 h after phlebotomy. In the first part of the study, TEGs on UFH-spiked, LMWH-spiked and DPD-spiked samples from the three control subjects were performed in parallel with an appropriate saline dilution at each of the six concentrations analysed. For the remaining seven subjects, UFH-spiked, LMWH-spiked and DPD-spiked samples at each concentration were analysed simultaneously. In the second part of the study, each concentration of anticoagulant was tested in standard and heparinase-coated TEG cuvettes in parallel. Tracings were recorded and analysis stopped once the tracing had reached the maximum amplitude, or after 60 min if a straight-line trace was produced. Clot retraction and fibrinolysis were not assessed in this study. TEG variables assessed were reaction time (R-time), clot formation time (K-time), clot formation rate (α -angle) and maximum amplitude (MA) (Fig. 1). Values of 60 min for the R-time and K-time, 0° for the α -angle and 0 mm for the MA were assigned arbitrarily to straight-line traces. Reference ranges for these variables were obtained from Medicell UK (for Haemoscope Corp., based on a 'normal population' mean \pm 2 SD), and were confirmed by our own in-house assessment of 20 normal subjects from the local population.

Fig. 1



Thromboelastogram (TEG) variables. The reaction time (R-time) is the time between activation of the sample with calcium, and the point where the amplitude of the tracing reaches 2 mm, representing the formation of the first fibrin strands. The clot formation time (K-time) is measured from this point until the amplitude reaches 20 mm, representing the time taken for a fixed degree of viscoelasticity to develop, as a result of fibrin build-up and crosslinking. The clot formation rate (α -angle) is that formed by the slope of the TEG tracing between the R-time and the K-time, and denotes the speed at which solid clot forms. The maximum amplitude (MA) is the greatest amplitude on the TEG trace and is a reflection of the absolute strength of the fibrin clot.

Other coagulation assays

In addition to a TEG, the PT, aPTT, TT, and anti-FXa activity were determined for each sample. A reptilase time was also performed on samples from three control subjects to demonstrate that abnormalities of coagulation in these samples were due to the addition of heparin and not an intrinsic abnormality of coagulation. Approximately 2.5 ml blood from each sample dilution was centrifuged at 1200 $\times g$ for 12 min at 4°C, the plasma aspirated and the sample re-centrifuged for a further 12 min. The supernatant plasma was frozen at -40°C until testing. Clotting times were assayed using standard techniques, with an IL-Futura Coagulation Analyser (Instrumentation Laboratories, Warrington, UK). Anti-FXa activity was determined using a chromogenic assay (Sigma Diagnostics, Sigma-Aldrich, Poole, Dorset, UK).

Statistical analysis

Descriptive statistics for all TEG and conventional test parameters are expressed as the mean \pm SEM. Linear regression analysis was used in both parts of the study to assess the trend in each TEG parameter or conventional coagulation test with increasing concentration of anticoagulant (against a hypothesis of no trend in each test, to a significance level of $P < 0.01$). Trend was determined because the power of the study for the determination of true linearity was limited.

In the first part of the study, the change in each TEG parameter versus concentration was analysed by calculating a summary slope for each parameter for each individual subject across the concentrations. The mean of these individual slopes was then tested for trend using a two-tailed paired t -test. Pearson's correlation coefficients were calculated to assess the correlation between concentration-dependent change in TEG parameters and conventional coagulation assays.

In the second part of the study, low concentrations of UFH, LMWH and DPD in the range 0.005–0.05 U/ml were assessed. The effect of heparinase on each TEG parameter at each concentration was evaluated using a paired two-tailed t -test. Mean summary slopes between heparinase-modified and standard TEG parameters were compared using a paired two-tailed t -test, in order to compare the sensitivity of differences between standard and heparinase-modified thromboelastography with the sensitivity of the anti-FXa assay for detecting changes between concentrations of UFH, LMWH and DPD. All calculations were performed with SAS for Windows version 8.02 and Microsoft Excel 2000.

Results

Table 1 presents the mean \pm SEM for each TEG parameter and conventional assay performed in 10 subjects for the first part of the study (concentrations of UFH, LMWH and DPD of 0.025–1.0 U/ml). The

Table 1 Comparison of thromboelastogram (TEG) parameters with conventional coagulation tests for detecting the presence of unfractionated heparin (UFH), low molecular weight heparin (LMWH; Fragmin) and danaparoid (DPD; Orgaran) (0.025–1.0 U/ml) in blood

Concentration (U/ml)	Saline control	UFH	LMWH	DPD
TEG parameters				
Reaction time (R-time) (9.4–27.4 min)				
0.025	14.2 ± 1.3	17.0 ± 1.4	17.8 ± 1.1	16.7 ± 2.0
0.050	13.8 ± 1.0	19.0 ± 1.9	17.2 ± 1.7	20.1 ± 4.1
0.100	12.7 ± 0.8	26.4 ± 1.8	15.9 ± 1.2	19.6 ± 2.1
0.250	15.0 ± 1.6	<u>46.0</u> ± 5.0	26.5 ± 3.4	27.0 ± 3.7
0.500	13.6 ± 1.5	<u>55.6</u> ± 4.4	<u>50.6</u> ± 5.5	<u>43.3</u> ± 4.4
1.000	14.2 ± 1.5	<u>60.0</u> ± 0.0	<u>59.3</u> ± 0.7	<u>56.7</u> ± 3.3
Clot formation time (K-time) (1.9–8.9 min)				
0.025	5.5 ± 0.4	7.1 ± 0.8	<u>9.8</u> ± 1.3	8.3 ± 1.5
0.050	5.2 ± 0.5	8.8 ± 1.0	<u>9.1</u> ± 1.8	8.9 ± 1.2
0.100	5.0 ± 0.5	<u>15.2</u> ± 1.6	<u>9.3</u> ± 1.3	<u>9.6</u> ± 1.9
0.250	5.2 ± 0.4	<u>41.7</u> ± 7.5	<u>19.1</u> ± 5.0	<u>17.2</u> ± 2.6
0.500	5.2 ± 0.5	<u>55.0</u> ± 5.0	<u>50.2</u> ± 6.6	<u>34.7</u> ± 6.5
1.000	4.5 ± 0.6	<u>60.0</u> ± 0.0	<u>60.0</u> ± 0.0	<u>56.4</u> ± 3.6
Clot formation rate (α-angle) (22–58°)				
0.025	39.9 ± 3.1	28.9 ± 3.1	24.1 ± 2.6	29.3 ± 4.7
0.050	40.1 ± 2.2	26.0 ± 3.1	25.7 ± 2.4	29.2 ± 3.7
0.100	41.2 ± 3.3	<u>16.6</u> ± 2.1	30.0 ± 2.4	27.5 ± 4.1
0.250	39.1 ± 2.9	<u>8.0</u> ± 3.5	<u>17.7</u> ± 2.2	<u>19.8</u> ± 3.2
0.500	39.6 ± 2.5	<u>2.0</u> ± 2.0	<u>6.0</u> ± 3.7	<u>9.8</u> ± 3.1
1.000	45.5 ± 2.8	<u>0.0</u> ± 0.0	<u>0.0</u> ± 0.0	<u>1.1</u> ± 1.1
Maximum amplitude (MA) (44.4–63.6 mm)				
0.025	48.3 ± 1.6	<u>43.6</u> ± 1.3	<u>41.8</u> ± 1.6	<u>43.9</u> ± 2.5
0.050	46.3 ± 1.6	<u>41.2</u> ± 1.6	<u>42.8</u> ± 2.1	<u>38.2</u> ± 3.7
0.100	50.1 ± 1.4	<u>40.3</u> ± 1.8	<u>42.7</u> ± 1.4	<u>43.1</u> ± 1.8
0.250	48.6 ± 1.2	<u>15.5</u> ± 5.3	<u>35.1</u> ± 4.5	<u>39.1</u> ± 2.3
0.500	47.8 ± 0.9	<u>3.7</u> ± 3.7	<u>9.2</u> ± 5.8	<u>24.0</u> ± 6.7
1.000	53.2 ± 1.2	<u>0.0</u> ± 0.0	<u>1.4</u> ± 1.4	<u>5.4</u> ± 5.4
Conventional coagulation parameters				
Prothrombin time (12–16 s)				
0.025	14.0 ± 0.2	14.2 ± 0.3	14.0 ± 0.3	14.22 ± 0.3
0.050	13.8 ± 0.2	14.2 ± 0.2	14.2 ± 0.3	14.34 ± 0.3
0.100	14.0 ± 0.2	14.8 ± 0.2	14.8 ± 0.6	14.44 ± 0.3
0.250	14.3 ± 0.3	15.9 ± 0.7	15.8 ± 0.7	14.79 ± 0.2
0.500	14.3 ± 0.3	18.0 ± 0.7	16.6 ± 0.4	16.04 ± 0.3
1.000	14.7 ± 0.3	<u>26.9</u> ± 2.4	<u>19.0</u> ± 0.4	<u>17.51</u> ± 0.3
Activated partial thromboplastin time (28–38 s)				
0.025	32.4 ± 0.8	33.2 ± 1.2	32.4 ± 2.0	33.5 ± 1.1
0.050	32.3 ± 0.7	34.3 ± 1.5	33.4 ± 2.2	35.8 ± 1.2
0.100	32.6 ± 1.0	41.1 ± 3.2	39.5 ± 3.6	37.5 ± 1.2
0.250	33.1 ± 0.7	<u>78.3</u> ± 13.4	<u>43.9</u> ± 2.1	<u>46.1</u> ± 1.7
0.500	33.6 ± 0.6	<u>130.4</u> ± 12.7	<u>66.5</u> ± 5.9	<u>59.2</u> ± 2.6
1.000	34.8 ± 0.8	<u>146.1</u> ± 14.2	<u>125.0</u> ± 10.0	<u>90.1</u> ± 5.6
Thrombin time (13–17 s)				
0.025	14.7 ± 0.4	15.6 ± 0.5	16.3 ± 1.3	14.7 ± 0.4
0.050	15.3 ± 0.5	16.7 ± 1.2	15.1 ± 0.4	15.1 ± 0.4
0.100	15.1 ± 0.3	<u>49.2</u> ± 5.6	<u>23.6</u> ± 4.3	15.0 ± 0.3
0.250	15.4 ± 0.6	<u>55.4</u> ± 4.6	<u>60.0</u> ± 0.0	<u>27.6</u> ± 5.6
0.500	15.2 ± 0.4	<u>60.0</u> ± 0.0	<u>60.0</u> ± 0.0	<u>30.5</u> ± 5.1
1.000	15.7 ± 0.4	<u>60.0</u> ± 0.0	<u>60.0</u> ± 0.0	<u>50.1</u> ± 5.1
Anti-FXa activity (U/ml)				
0.025	0.000 ± 0.000	<u>0.019</u> ± 0.007	<u>0.026</u> ± 0.006	<u>0.031</u> ± 0.010
0.050	0.000 ± 0.000	<u>0.049</u> ± 0.012	<u>0.053</u> ± 0.007	<u>0.081</u> ± 0.031
0.100	0.000 ± 0.000	<u>0.133</u> ± 0.024	<u>0.099</u> ± 0.008	<u>0.101</u> ± 0.013
0.250	0.000 ± 0.000	<u>0.321</u> ± 0.033	<u>0.211</u> ± 0.021	<u>0.302</u> ± 0.033
0.500	0.000 ± 0.000	<u>0.703</u> ± 0.043	<u>0.599</u> ± 0.043	<u>0.611</u> ± 0.036
1.000	0.000 ± 0.000	<u>0.894</u> ± 0.043	<u>0.924</u> ± 0.060	<u>0.925</u> ± 0.049

The mean ± SEM from 10 subjects with each drug are presented. anti-FXa, anti-activated factor X. Saline control values shown are the mean ± SEM of nine samples (three subjects, three drugs). The reference range for each parameter is shown in parentheses. Values outside the reference range are underlined. Straight-line traces were arbitrarily assigned reaction time and clot formation time values of 60 min, clot formation rate values of 0° and maximum amplitude values of 0 mm.

mean ± SEM of standard and heparinase-modified TEG parameters and conventional tests performed in 10 subjects from the second part of the study (concentrations of UFH, LMWH and DPD of 0.005–0.05 U/ml) are presented in Table 2. Values outside the normal range are underlined in both tables. In both parts of the study, the

addition of UFH, LMWH or DPD to citrated whole blood resulted in a prolongation of the R-time and K-time, and a reduction in the α-angle and MA of the standard TEG compared with control samples. The highest concentration of each anticoagulant (1.0 U/ml) produced a straight-line trace for up to 60 min,

Table 2 Comparison of standard and heparinase-modified thromboelastograph parameters with conventional coagulation tests for detecting the presence of low concentrations (0.05–0.005 U/ml) of unfractionated heparin (UFH), low molecular weight heparin (LMWH) and danaparoid (DPD) in blood

Concentration (U/ml)	UFH	UFH + heparinase	<i>P</i> <	LMWH	LMWH + heparinase	<i>P</i> <	DPD	DPD + heparinase	<i>P</i> <
Thromboelastograph parameters									
Reaction time (R-time) (9.4–27.4 min)									
0.005	16.4 ± 1.6	14.4 ± 0.9	0.2	16.0 ± 0.9	12.6 ± 1.1	0.001	15.4 ± 1.4	12.3 ± 1.3	0.01
0.010	16.5 ± 1.3	15.3 ± 1.3	0.2	17.8 ± 1.6	13.1 ± 0.8	0.01	16.8 ± 1.5	13.6 ± 1.1	0.05
0.025	18.5 ± 1.2	13.7 ± 1.1	0.001	18.7 ± 1.1	12.9 ± 0.9	0.001	17.2 ± 1.9	12.4 ± 1.2	0.01
0.050	21.7 ± 1.8	14.4 ± 1.6	0.001	18.7 ± 2.0	13.1 ± 0.6	0.02	21.8 ± 3.9	13.2 ± 1.1	0.05
Clot formation time (K-time) (1.9–8.9 min)									
0.005	7.1 ± 0.9	5.1 ± 0.6	0.05	<u>9.2 ± 1.1</u>	4.1 ± 0.6	0.01	8.2 ± 1.2	3.7 ± 0.5	0.01
0.010	6.7 ± 0.5	5.4 ± 0.5	0.01	<u>10.0 ± 1.4</u>	4.4 ± 0.5	0.01	<u>9.5 ± 1.5</u>	4.2 ± 0.4	0.01
0.025	8.8 ± 0.8	4.5 ± 0.6	0.01	<u>11.2 ± 1.1</u>	4.1 ± 0.5	0.001	<u>9.0 ± 1.3</u>	4.0 ± 0.7	0.01
0.050	<u>12.2 ± 1.4</u>	4.6 ± 0.6	0.001	<u>11.5 ± 2.1</u>	4.4 ± 0.4	0.01	<u>10.6 ± 1.0</u>	4.4 ± 0.7	0.001
Clot formation rate (α-angle) (22–58°)									
0.005	29.4 ± 2.5	38.3 ± 2.9	0.01	24.0 ± 2.2	45.6 ± 4.6	0.01	27.2 ± 3.1	48.3 ± 3.8	0.01
0.010	29.9 ± 2.1	37.1 ± 2.5	0.001	22.2 ± 2.4	44.4 ± 3.4	0.01	24.3 ± 2.9	44.3 ± 2.8	0.001
0.025	23.7 ± 1.9	40.9 ± 3.9	0.01	<u>20.0 ± 1.9</u>	46.4 ± 3.4	0.001	25.8 ± 4.1	46.3 ± 4.4	0.01
0.050	<u>18.6 ± 1.8</u>	41.6 ± 3.5	0.001	<u>21.8 ± 2.9</u>	42.2 ± 2.6	0.001	24.2 ± 3.4	43.8 ± 4.2	0.01
Maximum amplitude (MA) (44.4–63.6 mm)									
0.005	44.8 ± 2.0	47.7 ± 2.3	0.2	<u>40.0 ± 1.9</u>	51.1 ± 2.9	0.01	<u>42.6 ± 2.0</u>	51.5 ± 2.8	0.01
0.010	45.8 ± 1.2	50.1 ± 2.3	0.001	<u>40.6 ± 2.5</u>	51.5 ± 2.3	0.02	<u>42.6 ± 1.7</u>	51.5 ± 1.8	0.01
0.025	<u>43.1 ± 1.4</u>	51.8 ± 2.6	0.01	<u>40.0 ± 1.2</u>	53.5 ± 2.8	0.001	<u>42.5 ± 2.4</u>	50.9 ± 2.5	0.01
0.050	<u>41.0 ± 1.4</u>	50.7 ± 3.1	0.01	<u>39.5 ± 2.0</u>	51.3 ± 2.2	0.01	<u>36.5 ± 3.6</u>	51.1 ± 3.0	0.01
Concentration (U/ml)	UFH	LMWH	DPD						
Conventional coagulation parameters									
Prothrombin time (12–16 s)									
0.005	14.4 ± 0.3	14.3 ± 0.3	14.4 ± 0.4						
0.010	14.2 ± 0.3	14.4 ± 0.4	14.4 ± 0.3						
0.025	14.3 ± 0.3	14.3 ± 0.3	14.4 ± 0.3						
0.050	14.4 ± 0.3	14.5 ± 0.3	14.6 ± 0.4						
Activated partial thromboplastin time (28–38 s)									
0.005	33.8 ± 1.4	34.3 ± 1.4	33.5 ± 1.1						
0.010	33.6 ± 1.3	34.2 ± 1.3	33.3 ± 1.1						
0.025	34.4 ± 1.4	34.4 ± 1.4	35.0 ± 1.2						
0.050	35.4 ± 1.6	35.8 ± 1.6	37.4 ± 1.3						
Thrombin time (13–17 s)									
0.005	15.1 ± 0.5	14.5 ± 0.2	15.5 ± 0.7						
0.010	14.1 ± 0.2	14.2 ± 0.3	14.3 ± 0.3						
0.025	15.6 ± 0.5	16.3 ± 1.2	14.9 ± 0.4						
0.050	17.0 ± 1.1	15.4 ± 0.4	15.5 ± 0.4						
Anti-FXa activity (U/ml)									
0.005	0.034 ± 0.013	0.007 ± 0.004	0.004 ± 0.002						
0.010	0.034 ± 0.014	0.013 ± 0.005	0.009 ± 0.003						
0.025	0.044 ± 0.029	0.018 ± 0.006	0.029 ± 0.010						
0.050	0.052 ± 0.022	0.038 ± 0.010	0.083 ± 0.031						

The mean ± SEM from 10 subjects with each drug and paired *t*-test *P* values are presented. anti-FXa, anti-activated factor X. Values outside the reference range are underlined.

demonstrating complete inhibition of fibrin formation. TEG parameters were within the reference range for all saline control samples.

The results indicate that TEG parameters are modified by concentrations of UFH, LMWH and DPD that have no effect on standard PT, aPTT and TT assays. In the first part of the study, concentrations of all three anti-coagulants of less than 0.1 U/ml had no effect on PT, aPTT or TT assays, but caused a reduction in the MA of the TEG for all three anticoagulants (Table 1). The K-time was prolonged by LMWH at 0.025 U/ml, but not by UFH or DPD at this concentration. In the second part of the study examining low concentrations (0.005–0.05 U/ml) of UFH, LMWH and DPD, the PT, aPTT and TT were within the reference range for all

samples, but there was still a reduction in the MA of the TEG at UFH concentrations of 0.025 U/ml, and LMWH and DPD concentrations as low as 0.005 U/ml (Table 2). The K-time was prolonged by LMWH at concentrations of 0.005 U/ml, by DPD at 0.01 U/ml, and by UFH at 0.05 U/ml. Overall, the R-time and α-angle were the least sensitive of the TEG parameters in this study.

In the first part of the study, the variation in all four TEG parameters was concentration dependent over the range 0.025–1.0 U/ml, for all three anticoagulant drugs (*P* < 0.0001). In the second part of the study, the concentration-dependent variation in all four TEG parameters due to UFH over the range 0.005–0.05 U/ml was of borderline significance (*P* < 0.02), but changes in TEG parameters caused by LMWH or

DPD were not significant at these low concentrations. There was no correlation between TEG parameters and conventional clotting assays for all three anticoagulants across either of the concentration ranges studied (0.005–0.05 and 0.025–1.0 U/ml).

TEG parameters were within reference ranges for all tests performed in heparinase I-coated cuvettes in the second part of this study, suggesting that the enzyme had successfully neutralized the anticoagulant effect of all three drugs. The difference between standard and heparinase-modified TEGs was significant for all four parameters at the lowest concentration of LMWH and DPD studied (0.005 U/ml; $P < 0.01$) (Table 2). In contrast, only the difference between standard and heparinase-modified α -angles were significant for UFH at this concentration.

The difference between standard and heparinase-modified TEG parameters were further compared with the sensitivity of the anti-FXa assay for distinguishing between concentrations of all three anticoagulants over the range 0.005–0.05 U/ml. The difference between standard and heparinase-modified K-times and α -angles of the TEG were found to be more sensitive at distinguishing between concentrations of UFH across the range 0.005–0.05 U/ml than the anti-FXa assay ($P < 0.01$ versus $P = 0.43$). In contrast, the opposite was observed with LMWH and DPD over this concentration range ($P < 0.05$ for anti-FXa versus $P > 0.1$ for the TEG) (Table 3). The results suggest that, at low concentrations of UFH, the difference between standard and heparinase-modified TEG parameters may be a more sensitive method than the anti-FXa assay for detecting differences between small amounts of UFH in blood samples.

In the first part of the study, TEG parameters and conventional coagulation test results were within reference ranges for all saline control samples and showed limited inter-patient variability, as demonstrated by the relatively low standard error at each of the six 'concentrations' (saline dilutions) (Table 1). Because the same temporal sequence was used for testing all samples, the 'concentration' of each saline control was equivalent to time (starting with the 0.025 U/ml concentration tested 1 h after phlebotomy). The limited intra-subject variability shown by the saline

controls across the six 'concentrations' tested demonstrate that the clotting activity of citrated blood samples did not deteriorate significantly with storage for up to 7 h prior to analysis. These controls also confirmed that observed effects on the TEG or coagulation assays were not due to haemodilution from the addition of UFH, LMWH or DPD to blood samples. Reptilase times performed on anticoagulated blood from the three control subjects were also within the reference range (results not shown), demonstrating that changes in TEG and coagulation parameters for these samples were due to the addition of UFH, LMWH or DPD.

Finally, it is of note that LMWH and DPD significantly prolonged the aPTT at concentrations similar to those expected *in vivo* when prophylactic doses are administered subcutaneously (i.e. approximately 0.3 U/ml). UFH given subcutaneously might prolong the aPTT at this concentration, but prophylactic doses of LMWH should have little or no effect on the aPTT. This discrepancy probably results from the *in vitro* addition of these drugs to blood samples in what would be equivalent to an intravenous route of administration *in vivo*, and increased relative bioavailability of each anticoagulant due to the absence of endothelial cell binding.

Discussion

The TEG is increasingly used in surgery to detect global coagulation changes and guide blood product replacement peri-operatively. Most experience to date has been during hepatic and cardiac surgery [4,7,24,25], but the use of the TEG in other settings is increasing [26]. The observation from the present study that very low quantities of UFH, LMWH and DPD in whole blood have an effect on the TEG is significant, because many patients undergoing surgery receive some form of heparin therapy or prophylaxis pre-operatively [8,17]. Moreover, the implication from this and other studies is that even the quantities of heparin often used to flush intravenous catheters may affect the TEG [22,27]. Therefore, the reliability of the standard TEG as a guide for blood product replacement during surgery is limited by the possibility of heparin contamination.

It has been suggested that heparinase I-coated TEG cuvettes should be used for all samples where there is

Table 3 Mean difference ($n = 10$) between standard and heparinase-modified thromboelastograph summary slopes representing the change in each parameter versus anticoagulant concentration (0.005–0.05 U/ml), compared with the mean ($n = 10$) of summary slopes representing the change in anti-activated factor X (anti-FXa) activity versus anticoagulant concentration (0.005–0.05 U/ml)

Slope pairs (standard versus heparinase)	UFH		LMWH		DPD	
	Mean \pm SEM	$P <$	Mean \pm SEM	$P <$	Mean \pm SEM	P
Reaction time (R-time)	133.5 \pm 37.5	0.01	42.0 \pm 28.3	NS (0.2)	126.5 \pm 81.9	NS (0.2)
Clot formation time (K-time)	137.8 \pm 40.3	0.01	44.0 \pm 31.2	NS (0.2)	29.6 \pm 43.1	NS (0.51)
Clot formation rate (α -angle)	-352.0 \pm 104.0	0.01	22.9 \pm 80.5	NS (0.78)	23.6 \pm 182.9	NS (0.9)
Maximum amplitude (MA)	-179.8 \pm 65.1	0.05	-13.3 \pm 56.4	NS (0.82)	-126.5 \pm 100.4	NS (0.24)
Anti-FXa activity	0.3 \pm 0.4	NS (0.43)	0.7 \pm 0.2	0.02	1.8 \pm 0.7	0.05

a risk of heparin contamination [20]. Under conditions of endothelial stress such as surgery or sepsis, however, endogenous release of very small quantities of GAGs such as heparan sulphate occurs in some patients [28]. There is also evidence that these GAGs can influence the TEG, an effect that is reversed by the use of heparinase I-coated TEG cuvettes [2,11,14,20]. These findings are supported by the present study, where TEG changes due to minute quantities of DPD (a mixture of GAGs including heparan sulphate) were completely reversed by heparinase I. Endogenously released GAGs may be clinically significant in terms of an increased bleeding risk for some patients [2,11]; therefore eliminating this effect on the TEG with heparinase and disregarding it as heparin contamination may ignore some clinically important information regarding blood coagulation. These limitations of the standard and heparinase-modified TEG make it very difficult to standardize the assay or to produce reliable guidelines for blood product replacement during surgery. Therefore, both standard and heparinase-modified TEGs should be performed in parallel in all patients where there is a risk of heparin or GAG contamination, but the results should be cautiously interpreted in the context of each individual clinical setting.

Inhibition of coagulation by UFH, LMWH and DPD as measured by the TEG was shown to be concentration dependent over the range 0.025–1.0 U/ml, which is in concordance with the results of earlier studies [9,29]. However, the current study is the first to assess the effects of concentrations of UFH, LMWH and DPD below 0.1 U/ml. While standard TEG changes due to UFH were significantly concentration dependant between 0.005 and 0.05 U/ml, this was not true for LMWH and DPD. In addition, the differences between standard and heparinase-modified TEG parameters were shown to be more sensitive to very low quantities of UFH than anti-FXa activity, but less sensitive to LMWH and DPD. The variable sensitivity of TEG parameters and conventional coagulation assays to the presence of low concentrations of UFH, LMWH and DPD may reflect the different composition and mechanisms of action of these anticoagulants. UFH acts via potentiation of antithrombin in the inhibition of thrombin and factor-FXa [30], whereas LMWH predominantly inhibits factor-FXa [31]. Both UFH and LMWH are renally excreted, but, unlike LMWH, UFH also binds non-specifically to plasma proteins and macrophages, accounting for the variable in-vivo activity that is often observed after administration of UFH to patients [32]. Danaparoid is a mixture of 84% heparan sulphate, 12% dermatan sulphate and 4% chondroitin sulphate. While heparan sulphate acts via antithrombin in a similar manner to UFH and LMWH [33], dermatan sulphate has a different mechanism of anticoagulation via potentiation of heparin cofactor II [34], and chondroitin sulphate is thought to be devoid of any anticoagulant activity [9].

Zmuda *et al.* commented that concentrations of enoxaparin (LMWH) and DPD resulting in 'minor' prolongations of the aPTT (< 10 s) markedly or completely inhibited coagulation as measured by the TEG [9]. The results of the current study support these findings, and similar observations have been made in a recent in-vitro study of the effects of fondaparinux and enoxaparin on tissue factor-triggered thromboelastography [29]. Since the generation of the TEG is multifactorial and involves the entire blood coagulation system including platelets and fibrinogen [7,15], one may have expected the TEG to be less sensitive to UFH, LMWH and DPD than the aPTT, because the aPTT analyses only a part of the coagulation cascade in isolation.

In the current study there was no significant correlation between concentration-dependent changes observed in TEG parameters compared with conventional coagulation assays, which is in contrast to the findings of previous in-vivo studies [8,12,14]. The absence of a correlation in the current study may reflect the in-vitro addition of anticoagulants to blood samples and the wide range of concentrations of UFH, LMWH and DPD that were used (0.005–1.0 U/ml), since changes in the TEG parameters observed at low concentrations of each anticoagulant were not detected by the PT, aPTT and TT assays.

Blood samples require up to 60 min standing time before processing in order to obtain reproducible TEG results [10,23]. In addition, the large number of anticoagulant concentrations tested per subject in this study and the duration of each TEG (up to 60 min) resulted in storage of blood in citrate for a significant period of time. A number of studies have investigated whether citrate storage significantly affects the TEG. Most agree that citration of blood does influence the TEG, but there are conflicting data as to whether these changes are stable and lead to reproducible results. Roche *et al.* [35] compared TEG parameters obtained by native blood, citrated blood stored at room temperature and citrated blood that had been refrigerated. They found that citration and recalcification of blood 'pre-activated' the sample, and that refrigeration increased the magnitude of this effect. Other studies performed on blood from patients with liver disease [13] or undergoing surgery [36] have demonstrated that blood stored in citrate remains stable and produces reliable TEG tracings between 1 and 8 h after phlebotomy. These findings are supported by our study, where TEG parameters were preserved within the reference range for citrated saline control samples stored for up to 7 h before analysis. However, such arguments are more important for standardization of the TEG for use in clinical practice than to the results of the present in-vitro study, where similar concentrations of each anticoagulant were compared in the same temporal sequence.

In conclusion, the results demonstrate that TEG parameters are modified by concentrations of UFH, LMWH and DPD that have no effect on conventional fibrin-based PT, aPTT and TT assays. Calculation of the difference between standard and heparinase-modified TEG parameters greatly increases the sensitivity of the TEG for the effects of UFH, LMWH and DPD, and is more sensitive to very low quantities of UFH than anti-FXa activity.

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