A cross-over comparison of the anti-clotting effects of three low molecular weight heparins and glycosaminoglycuronan

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- 1 The anti-clotting effects after intravenous administration of three low molecular weight (LMW) heparins, Fragmin[®] (KABI 2165), Fraxiparine[®] (CY 216), Clexane[®] (PK 10169) and the LMW mixture of glycosaminoglycuronans Orgaran[®] (Org 10172) were compared in a randomized cross-over study in 12 healthy male volunteers.
- 2 The time courses of the anti-Xa activity of Fragmin[®], Fraxiparine[®] and Clexane[®] (five subjects) were best fitted by a monoexponential function and had comparable half-lives of 1.9 h, 2.3 h and 2.8 h, respectively. The time courses of the anti-Xa activity of Orgaran[®] and Clexane[®] (four subjects) were described by a biexponential function with terminal half-lives of 56.8 h and 27.7 h, respectively. They were longer than those of Fraxiparine[®] and Fragmin[®]. Orgaran[®] injection was associated with a significantly smaller 'clearance' ($0.8 \pm 0.2 \text{ l h}^{-1}$) of the plasma anti-Xa activity compared with Fragmin[®] (2.0 ± 0.5), Fraxiparine[®] (1.7 ± 0.5) and Clexane[®] (1.6 ± 0.5).
- 3 In comparison with the three LMW heparins, the terminal half-life of plasma anti-IIa activity after Orgaran[®] was longer and the 'clearance' of Orgaran[®] was lower than that after Clexane[®]. The area under the curve of the plasma anti-IIa activity after administration of Orgaran[®] was negligible compared with that obtained after injection of the LMW heparins.
- 4 Orgaran[®] caused the smallest and Fragmin[®] the greatest prolongation of the activated partial thromboplastin time (Orgaran[®] $5.8 \pm 1.2 \text{ s}$ vs Fragmin[®] $18.5 \pm 5.2 \text{ s}$) and the thrombin clotting time (Orgaran[®] $2.9 \pm 1.7 \text{ s}$ vs Fragmin[®] $47.8 \pm 0.9 \text{ s}$).
- 5 The LMW heparins clearly differ kinetically from each other and from the low molecular weight compound Orgaran[®]. Until the clinical importance of these differences is known, the low molecular weight heparins cannot be considered as an undifferentiated group of drugs.

Keywords Orgaran[®] low molecular weight heparins

Introduction

Low molecular weight heparins have been developed to improve the benefit-risk ratio of heparin. The new LMW heparins and Orgaran[®], a mixture of glycosaminoglycuronans derived from porcine intestinal mucosa, differ from standard heparin in several respects. Thus, they produce a greater plasma anti-Xa/anti-IIa ratio *in vitro* and have less effects on *in vitro* platelet aggregation. As a consequence of the more selective effects on the coagulation cascade they cause less prolongation of the activated partial thromboplastin time (APTT) and thrombin clotting time (TCT) and marked divergence of the time courses of plasma anti-Xa and anti-IIa activities

[1, 2, 3, 4]. In animal studies Orgaran[®] had antithrombotic activity with only a small dose-dependent bleeding enhancing capacity compared with standard heparin at doses causing similar antithrombotic effects [5].

All drugs used in this study have been shown to be effective in the prophylaxis of deep venous thrombosis (DVT) and in one report a LMW heparin was found to be more effective than heparin, while the bleeding risk was reduced [6]. The half-lives of plasma anti-Xa activity after LMW heparins and Orgaran[®] in healthy volunteers were found to be considerably longer than that after standard heparin and they show improved bioavailability

with respect to effect on clotting factors after subcutaneous administration [2, 3, 7]. The time-curves of the effects of different low molecular weight heparins and Orgaran® have been described previously but comparisons are difficult because different assays and reference standards have been used [8]. No studies have been reported comparing LMW heparins with Orgaran®. The present cross-over study was performed to compare the time-courses of plasma anti-Xa, anti-IIa, and thrombin generation inhibiting activity and the effect on the APTT and the TCT after intravenous bolus doses of Fragmin®, Fraxiparine®, Clexane® and Orgaran®.

Methods

Medication

Fragmin® is produced by partial depolymerisation of heparin by nitrous acid hydrolysis. The mean molecular weight is about 5000 daltons. Clexane® is obtained by depolymerisation of the benzylic ester of porcine mucosal heparin. Clexane® has a molecular weight range from 3000 to 10000 (mean 5000) daltons. Fraxiparine® is produced by depolymerisation and ethanol precipitation of commercially available heparin. It has a molecular weight range from 2000 to 8000 (mean 4500) daltons. Orgaran® is derived from animal porcine intestinal mucosa by methanol precipitation and ultrafiltration. Orgaran® has a molecular weight range from approximately 4000 to 10000 (mean 6000) daltons.

Subjects

Twelve healthy male volunteers aged 20 to 27 years and weighing 63 to 87 kg (mean $\pm$ s.d.: 77 ± 6 kg) were recruited after having given informed consent. All were devoid of bleeding diathesis and had normal prothrombin time (normal value < 14.5 s), APTT, TCT, and bleeding time values (Simplate®, normal value < 9 min). The volunteers were admitted to the research unit, approximately 1 h prior to starting the study treatment and remained under observation for 24 h after Fragmin®, Fraxiparine® and Clexane® administration and for 48 h after Orgaran® administration.

Protocol

The volunteers received an intravenous infusion of each of the three LMW heparins and Orgaran® over a period of 1 min according to an open randomized cross-over design. Treatment order was determined by three Latin squares (4×4). The administration of treatments was separated by a washout period of at least 1 week. The doses given in this study were based upon the once daily subcutaneous dosage recommendation for high risk DVT patients for Fragmin®, Fraxiparine®, and Clexane®. To enable more precise evaluation of the time-course of effects, the Orgaran® dose used in this study was 2.5 times higher than the recommended total daily subcutaneous dose for prophylaxis of DVT. The declared specific activities as well as the anti-Xa, anti-IIa and IIaGI activities of each test substance measured with the

Table 1 Declared and measured amidolytic activities (iu) of the LMW heparins and Orgaran®

	Declared	Measured		
		anti-Xa	anti-IIa	IIaGI
Fragmin®	5000 anti-Xa	5331 ¹	2199 ¹	5261 ³
Fraxiparine®	7500 I.C.*	3457 ¹	789 ¹	2786 ³
Clexane®	40 mg	4887 ¹	1215 ¹	3171 ³
Orgaran®	3750 anti-Xa	3728 ²	132 ²	2520 ³

¹ Measured activities against WHO LMW standard.

² Measured activities against Orgaran® standard.

³ Measured activities by IC₅₀ value assessment and expressed as anti-Xa equivalent units.

* Institute Choay anti-Xa units.

assays used in this study are shown in Table 1. When necessary the LMW heparins and Orgaran® solutions were diluted with saline to a volume of 4.0 ml of which 3.0 ml was infused over a period of 1 min in order to administer a dose of 5000 anti-Xa units of Fragmin®, 7500 Institute Choay units (3075 IU anti-Xa) of Fraxiparine®, 40 mg of Clexane® (4000 IU anti-Xa), and 3750 anti-Xa units of Orgaran®. Sequential blood samples for routine haematological and coagulation measurements were taken for evaluation of the time-curves of effects up to 72 h. The amount of anti-Xa activity in the urine was measured from 0–6 h and from 6–24 h and expressed as the percentage of the administered dose of anti-Xa activity. The study protocol was approved by the Ethics Committee of Leiden University Hospital.

Assay methods

Activated partial thromboplastin time (APTT) and thrombin clotting time (TCT) were measured by standard procedures [9]. The plasma samples were snap frozen and thawed at 37° C just prior to assessment of the anti-coagulant activity. Plasma anti-Xa activity was measured by amidolytic microtitre plate assay with the chromogenic substrate S2222 (Kabi Diagnostics, Stockholm, Sweden), using a modification of the method of Teien & Lie [10]. For measurement of urine anti-Xa activity the same amidolytic microtitre plate assay was used with the chromogenic substrate S2222 but with the addition of antithrombin-III. The urine and plasma anti-Xa activities after administration of Fragmin®, Fraxiparine® or Clexane® were calculated from a standard curve prepared with the first international standard (1987) for LMW heparin. Plasma anti-Xa activities after administration of Orgaran® were calculated from a standard curve prepared with the Orgaran® in-house standard which was calibrated against the international heparin standard in a buffer system. Plasma anti-IIa activity was measured using an amidolytic microtitre plate assay with the chromogenic substrate S2238 (Kabi Diagnostics) [11]. The anti-IIa activities after administration of Fragmin®, Fraxiparine® or Clexane® were calculated from a standard curve prepared with the first international standard (1987) for LMW heparin, whereas the plasma anti-IIa activities after administration of Orgaran® were calculated from a standard curve prepared with the Orgaran® standard calibrated against the international heparin standard in a buffer system. Plasma thrombin-

generation inhibiting activity (IIaGI) was measured by a two-step thrombin generation inhibition assay modified from Ofosu *et al.* [12]. The amount of thrombin generated from prothrombin after activation by the prothrombinase complex was assessed with the chromogenic substrate S2238 (Kabi Diagnostics). The IIaGI activities were calculated using standard curves for each test drug prepared in plasma from individual subjects and expressed as equivalent anti-Xa units.

Data analysis

Individual pre-drug (baseline) plasma anti-Xa and anti-IIa levels were subtracted from subsequent individual levels prior to estimation of kinetic parameters. As plasma samples taken before drug administration were used for the IIaGI calibration curve for each volunteer, pre-drug IIaGI values were considered zero by definition. Plasma anti-Xa, anti-IIa and IIaGI levels below the limits of quantitation, defined as three times the s.d. of a series of measurements in blank plasma, were not used for analysis. When samples contained measurable amounts at times after the limit of quantitation had been reached, these were disregarded. The parameters of mono- or bi-exponential functions describing the time-courses of plasma anti-Xa, anti-IIa and IIaGI activities were obtained using the non-linear least squares program SIPHAR (SIMED, Creteil, France). Model parameters were used for statistical analysis only if acceptable fits to the data could be obtained. Area under the curve (AUC) values for activities in plasma were calculated by the linear trapezoidal rule using values from zero time to the last data point. 'Clearance' (CL) of the activities was calculated from the ratio of administered dose/AUC of plasma activity. Terminal half-lives of activities were determined from $\ln 2/\lambda_z$, where λ_z is the terminal elimination rate constant obtained from the fitted function. 'Renal clearance' of activity was calculated

from the ratio of urinary recovery/AUC of plasma activity. Variables were subjected to analysis of variance with treatment (four levels), period (four levels) and subject (twelve levels) as fixed factors.

To evaluate the effect of treatments, 95% confidence intervals (CI) on differences between treatments (δ) were calculated. All analyses were carried out with the procedure GLM of the SAS package [13].

Results

The i.v. administration of all four test drugs was well tolerated. No pain or discomfort was reported. No signs of bruising or bleeding were observed except for minor bruises at the site of insertion of the cannula for venous blood sampling. The subjects did not report any clinically relevant adverse events during the 48 h observation period following the injection. One subject experienced minor discomfort during the Clexane® injection at the site of the indwelling venous cannula. It was concluded from the shape of the time course of the plasma anti-Xa activity in this individual, that some of the dose had been injected extra-vascularly. These data were excluded from the statistical analysis.

Parameters describing the time-courses of plasma anti-Xa, plasma anti-IIa and plasma IIaGI are summarised in Table 2 and mean data for each treatment are shown in Figures 1, 2 and 3.

The time courses of plasma anti-Xa effects after administration of Fragmin® and Fraxiparine® were described by a monoexponential function, whereas for Orgaran® a biexponential function was appropriate. Four of the twelve curves measured after administration of Clexane® were best fitted by a biexponential function and five by a monoexponential function. Two data sets were excluded from analysis because it was not possible

Table 2 Kinetic parameters (mean $\pm$ s.d.) describing the time-course of plasma anti-Xa, anti-IIa and IIaGI effect, the % recovered anti-Xa activity in urine and the prolongation of APTT and TCT 1 hour after administration

		Fragmin®	Fraxiparine®	Clexane®	Orgaran®
Anti-Xa:	$t_{1/2,z}$ (h) (monoexponential)	1.9 $\pm$ 0.3	2.3 $\pm$ 0.4	2.8 $\pm$ 0.4	
	$t_{1/2,z}$ (h) (biexponential)			56.8 $\pm$ 23.3**	27.7 $\pm$ 15.4*
	$t_{1/2}$, initial (h) (biexponential)			2.1 $\pm$ 0.5	3.2 $\pm$ 1.0
	'CL' (l h ⁻¹)	2.0 $\pm$ 0.5	1.7 $\pm$ 0.5	1.6 $\pm$ 0.5	0.8 $\pm$ 0.2*
Anti-IIa:	$t_{1/2}$ (h)	1.4 $\pm$ 0.3	1.5 $\pm$ 0.2	1.3 $\pm$ 0.2	2.0 $\pm$ 0.9*
	'CL' (l h ⁻¹)	3.1 $\pm$ 0.9	2.9 $\pm$ 0.9	4.1 $\pm$ 0.1**	2.5 $\pm$ 1.5*
IIaGI:	$t_{1/2,z}$ (h)	1.2 $\pm$ 0.2	1.3 $\pm$ 0.1	1.1 $\pm$ 0.2	3.3 $\pm$ 0.9*
	$t_{1/2}$, initial (h)				0.4 $\pm$ 0.2
	'CL' (l h ⁻¹)	2.8 $\pm$ 0.7	2.9 $\pm$ 1.0	2.8 $\pm$ 0.8	2.1 $\pm$ 0.5*
Urine:	% recovered 0–6 h	10.6 $\pm$ 1.0	9.7 $\pm$ 1.9	9.2 $\pm$ 0.9	14.3 $\pm$ 1.6*
	% recovered 6–24 h	0.8 $\pm$ 0.8	0.7 $\pm$ 0.5	1.7 $\pm$ 0.3	5.1 $\pm$ 1.6*
	'renal CL' (ml min ⁻¹)	4.1 $\pm$ 0.6##	3.5 $\pm$ 1.2	3.3 $\pm$ 1.0	3.3 $\pm$ 0.7
Coag. test:	Δ APTT (s)	18.5 $\pm$ 5.2##	7.5 $\pm$ 3.8	7.7 $\pm$ 2.8	5.8 $\pm$ 1.2
	Δ TCT (s)	47.8 $\pm$ 0.9##	17.8 $\pm$ 13.2	24.7 $\pm$ 11.9	2.9 $\pm$ 1.7*
Ratio:	anti-Xa/anti-IIa	3.9 $\pm$ 0.9	7.7 $\pm$ 2.2	12.0 $\pm$ 6.0	90.3 $\pm$ 51.2

* = Significantly different from Fragmin®, Fraxiparine® and Clexane® ($P < 0.05$)

** = Significantly different from Fragmin®, Fraxiparine® and Orgaran® ($P < 0.05$)

= Significantly different from Clexane® ($P < 0.05$)

= Significantly different from Fraxiparine®, Clexane® and Orgaran® ($P < 0.05$).

Figure 1 Time courses of the mean plasma anti-Xa activity.
● = Fragmin®, ○ = Fraxiparine®, ▲ = Clexane®,
△ = Orgaran®.

Figure 2 Time courses of the mean plasma anti-IIa activity.
● = Fragmin®, ○ = Fraxiparine®, ▲ = Clexane®,
△ = Orgaran®.

Figure 3 Time courses of the mean plasma IIaGI activity.
● = Fragmin®, ○ = Fraxiparine®, ▲ = Clexane®,
△ = Orgaran®.

to obtain adequate fits. Orgaran® was characterized by a significantly smaller plasma 'clearance' of the anti-Xa activity compared with Fragmin® ($\delta = 1.2$ CI:0.8,1.4 u ml⁻¹), Fraxiparine® ($\delta = 0.9$ CI:0.6,1.2 u ml⁻¹) and Clexane® ($\delta = 0.8$ CI:0.4,1.1 u ml⁻¹).

The time-courses of plasma anti-IIa activity after

administration of all four test drugs were best described by monoexponential functions. All pre-drug plasma anti-IIa values were zero with the exception of one value (0.01 u ml⁻¹). The half-life of plasma anti-IIa activity after Orgaran® was significantly longer than those of Fraxiparine® ($\delta = 0.5$ CI:0.0,1.0 h), Fragmin® ($\delta = 0.6$ CI:0.1, 1.0 h) and Clexane® ($\delta = 0.7$ CI:0.3,1.2 h). The 'clearance' of plasma anti-IIa activity after Clexane® was significantly greater than that for Fraxiparine® ($\delta = 1.2$ CI:0.5,1.9 u ml⁻¹), Fragmin® ($\delta = 1.0$ CI:0.3,1.7 u ml⁻¹) and Orgaran® ($\delta = 1.6$ CI:0.9,2.3 u ml⁻¹). Compared with the LMW heparins, Orgaran® induced only a minimal and short-lasting plasma anti-IIa effect (Figure 2). After the relatively high dose of Orgaran® the peak plasma anti-IIa value was only just above the limit of quantitation of the assay. Consequently the limit of quantitation (0.02 anti-IIa u ml⁻¹) was achieved within 2 h after Orgaran® administration in almost all subjects, whereas this value was reached at 3–6 h after administration of the three LMW heparins.

The time courses of IIaGI effect obtained after Fragmin®, Fraxiparine® and Clexane® were best described by monoexponential functions whereas that after Orgaran® was described by a biexponential function. After administration of Orgaran® the half-life of plasma IIaGI activity was longer than that of Fragmin® ($\delta = 1.2$ CI:1.6,2.4 h), Fraxiparine® ($\delta = 2.0$ CI:1.5,2.4 h) and Clexane® ($\delta = 2.1$ CI:1.7,2.6 h) and the 'clearance' of Orgaran® was reduced in comparison with Fragmin® ($\delta = 0.7$ CI:0.3,1.1 u ml⁻¹), Fraxiparine® ($\delta = 0.7$ CI:0.3,1.2 u ml⁻¹) and Clexane® ($\delta = 0.6$ CI:0.2,1.1 u ml⁻¹). No significant differences were seen between the three LMW heparins with regard to the time-course of IIaGI. Plasma IIaGI values 15 min after administration of Orgaran® were lower than those after Fragmin®, or Clexane®, but higher than those after Fraxiparine®. However, 8 h after the administration of Orgaran® IIaGI values were still detectable at levels ≥ 0.02 anti-Xa equivalent units, whereas 5–6 h after administration of each of the LMW heparins the IIaGI values were below the limit of quantitation.

The urinary recovery of anti-Xa activity after Orgaran® was significantly greater than that after administration of the three LMW heparins for both collection periods. However, the 'renal clearance' of activity after Fragmin® was significantly greater than that after administration of each of the other products (Table 2).

The administration of Fraxiparine®, Clexane® and Orgaran® was associated with similar mean prolongations of the APTT after 1 hour: 7.5 s, 7.7 s, and 5.8 s, respectively (Table 2). However, Fragmin® prolonged the APTT by 18.5 s, a significantly longer time. Six hours after administration of each of the four drugs the APTT had returned to baseline or near baseline values. The mean prolongation of the TCT 1 h after Orgaran® administration was significantly less than after the other LMW heparins. By contrast, Fragmin® caused a considerable prolongation of the TCT at that time, which was significantly greater than that after the other substances. The mean prolongations of the TCT 1 h after Fraxiparine® and Clexane® were between those following Orgaran® and Fragmin®. The TCT had returned to baseline values 6 h after administration of each of the four drugs.

Discussion

Most of the kinetic parameters estimated in this study were comparable with those reported in previous studies of the individual LMW heparins and of Orgaran® [1,2,3,4]. However, in some subjects the kinetics of clotting factor activities after Clexane® differed from earlier reports [14]. In those subjects in whom the anti-Xa data were described by a biexponential function a longer terminal half-life was observed than described previously. It is likely that our use of a more sensitive assay allowed measurements of low plasma anti-Xa values for a longer period of time. The distribution half-life of Clexane® effect (2.1 ± 0.5 h) is therefore probably comparable with the value observed in those subjects whose data were described by a monoexponential function.

Orgaran® differs markedly from the LMW heparins in its kinetics. Thus, it is characterized by significantly smaller 'clearances' of plasma anti-Xa activity and plasma IIa generation inhibition and a longer half-life compared with Fragmin® and Fraxiparine®. If the inhibition of the generation of thrombin in plasma contributes significantly to the prevention of DVT, then the kinetic data from this study supports a multiple rather than a once a day dosing scheme for all of the compounds studied [15]. The virtual absence of plasma anti-IIa activity after intravenous administration of 750 anti-Xa units Orgaran® (the recommended twice a day prophylactic s.c. dose for deep venous thrombosis) contrasts with the effect of the three LMW heparins. Thus, the kinetic data suggest that the duration of effects after once a day dosage of each of the three LMW heparins is too short for optimum antithrombotic effect. After an i.v. dose of 2.5 times the recommended total 24 h dose, Orgaran® caused a moderate prolongation of the APTT and only a minimal prolongation of the TCT. The observed prolongations in the TCT closely reflected the measured plasma anti-IIa activity at 1 h for all test substances. However, for the determination of APTT and TCT only one blood sample (at 1 h) was taken near the peak effects on these parameters. It is emphasized therefore, that differences in the integrated effects on these parameters over time

may be less. The plasma 'clearance' of anti-Xa activity after Orgaran® was lower than after the three LMW heparins. Although there were no major differences in 'renal clearance', 19% of the activity of Orgaran® was cleared by the kidney over 24 h compared with 11% after Fragmin®, Fraxiparine® and Clexane®. Differences in 'renal clearance' cannot explain the differences in anti-Xa activity between the substances used.

In this study the preparations were given intravenously and the findings may not reflect the situation after clinical administration by subcutaneous injection. The high anti-Xa/anti-IIa ratio suggests a different 'clearance' of these two activities [16]. In this study the differences in the anti-Xa/anti-IIa ratio (Table 2) between the four drugs are reflected in their effects on APTT and TCT. However, Fragmin®, which has a greater ratio than unfractionated heparin, has comparable effects to heparin on APTT and TCT. Therefore the value of this ratio may be questioned. The anti-Xa/anti-IIa ratio for Orgaran® is very high, owing to its low intrinsic anti-IIa activity. The plasma anti-IIa activity values after treatment with this compound were close to the limit of quantitation.

The clinical significance of the observed differences in kinetic parameters is uncertain as none of the plasma amidolytic effects studied have yet been shown to correlate directly with antithrombotic or bleeding effects in man. The recently reported reduction of the increased anti-Xa/anti-IIa ratio in the presence of Ca^{2+} ions further complicates interpretation with regard to the clinical significance of the differences in plasma effect kinetics [17]. A randomized, blind comparison of the clinical effects and the bleeding enhancing potential of these new drugs, together with accurate analyses of their amidolytic plasma responses, may contribute to a better insight into any correlation between clinical and the plasma amidolytic effects and may facilitate the design of optimum dosing schedules. The LMW heparins clearly differ kinetically from each other and from the low molecular weight compound Orgaran® and produce effects that vary differently with time. Until the clinical importance of these differences is known, the low molecular weight heparins cannot be considered as an undifferentiated group of drugs.

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