Bioequivalence of subcutaneous and intravenous body-weight-independent high-dose low-molecular-weight heparin Certoparin on anti-Xa, Heptest, and tissue factor pathway inhibitor activity in volunteers


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The objective of the study was to demonstrate the bioequivalence of subcutaneously (s.c.) and intravenously (i.v.) administered fixed, high-dose low-molecular-weight heparin (LMWH) on anti-activated factor X activity (anti-FXa). Secondary objectives were the analysis of the pharmacodynamic effects on Heptest, thrombin inhibition, tissue factor pathway inhibitor (TFPI), and the urinary excretion of LMWH in the randomized cross-over study following i.v. and s.c. application of 8000 anti-FXa units LMWH Certoparin in 18 healthy subjects. The bioequivalence following s.c. administration was demonstrated from the antilog of the point estimator for the application differences (s.c. minus i.v.) by an area under the activity–time curve (0–24 h) of 101% (range, 93–110%). LMWH was bioequivalent also on Heptest and TFPI, and was 50% on thrombin inhibition. The urinary excretion of biologically active material was 4.1% and 3.6% following i.v. and s.c. administration, respectively. Differences in the pharmacodynamic parameters of the assays indicate specific biological actions of high and low molecular saccharide chains of the LMWH. Blood Coagul Fibrinolysis 13:1–8 © 2002 Lippincott Williams & Wilkins.

Keywords: low molecular weight heparin, bioequivalence, factor Xa, pharmacokinetic, pharmacodynamic

Introduction

Low-molecular-weight heparins (LMWHs) were investigated first for the prevention of venous thromboembolism in patients at risk [1]. The positive results of the primary prevention suggested their usage in the treatment of acute venous thromboembolism as well. Many comparative studies demonstrated an equal or superior efficacy of body-weight-adjusted subcutaneous (s.c.) LMWHs compared with activated partial thromboplastin time (aPTT)-controlled intravenous (i.v.) unfractionated heparin (UFH) in the treatment of acute deep venous thrombosis [2–5]. This was demonstrated also for a twice-daily, subcutaneous, body-weight-independent high-dose regimen of LMWH [6]. Pharmacokinetic and pharmacodynamic studies showed an enhanced half-life and a complete bioavailability of LMWHs towards activated factor X (FXa) compared with normal heparin in man [7–10]. The studies included comparisons of individual LMWHs with UFH, different dosages of LMWH,
analysis of bioequivalence, and urinary excretion of radiolabelled or biologically active material in healthy persons and patients with impairment of renal function or myocardial infarction [11–13]. All studied patients adopted fixed low doses or body-weight-adjusted high doses of LMWHs. Pharmacokinetic and pharmacodynamic parameters of some LMWHs have been analysed in detail [10,14]. All results indicated that the individual pharmacological characteristics differ between the LMWHs.

Certoparin is a LMWH obtained by nitrous acid degradation of unfractionated pig intestinal mucosa heparin. The mean molecular weight is 5600 Da, compared with 15 000 Da of the original compound, and the ratio for the inhibition of FXa to thrombin in vitro is 2 compared with 1 for UFH.

A fixed, body-weight-independent dose of Certoparin is developed for treatment of acute deep venous thrombosis. Pharmacokinetic and pharmacodynamic studies are available only for body-weight-adjusted s.c. LMWH. LMWH is distributed mainly, if not at all, in the intravascular space, varying less between individuals than the body weight. The fixed dosage of i.v. and s.c. LMWH should therefore result in a low inter-individual variability of the pharmacodynamic parameters. We also analysed the bioequivalence of i.v. and s.c. LMWH on different coagulation enzymes to investigate differences of the mode of action in vivo in relation to the low and high molecular weight fractions of LMWH.

Materials and methods

Study design

Eighteen male healthy volunteers aged 22–36 years participated in the single-dose, randomized, and two period cross-over studies to receive 8000 IU of the LMWH Certoparin (Novartis Pharma GmbH, Nuremberg, Germany) intravenously and subcutaneously at weekly intervals. No concomitant medications were permitted other than those to treat potential adverse events. The study was conducted according to the principles of the Declaration of Helsinki and the Note for Guidance on Good Clinical Practice. The local ethical committee accepted the study protocol. Subjects gave written informed consent prior to entering the study.

Eligible subjects were admitted to the study centre 2 h before Certoparin and were discharged after completing the 24 h assessments. Each subject underwent a full physical examination prior to receiving Certoparin. In addition, the medical history, body weight, height, vital signs, and a 12-lead electrocardiogram were recorded. The presence of occult blood in faeces and urine was assessed, and routine clinical tests were conducted. Screening procedures were repeated at 24 h after every administration. Pathological results should be repeated within 24 h intervals until normalized.

Blood sampling

Free flowing blood was taken from a plastic canula inserted into the cubital vein by clean venipuncture and was anticoagulated with 3.8% sodium citrate. Samples were drawn before and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 8, 12, and 24 h after i.v. or s.c. administration of 8000 IU Certoparin. Blood samples were centrifuged immediately at 1500 × g and 4°C for 10 min. The midstream urine was collected 0, 4, 8, 12, and 24 h after application of Certoparin in both treatment groups. The aPTT and the Heptest clotting assay were performed within 30 min. For the measurement of the anti-activated factor X (anti-FXa) activity in urine, thrombin clotting time (TCT), tissue factor pathway inhibitor (TFPI) and anti-factor II activity, the samples were shock frozen and stored at −80°C until analysis. These parameters were determined blindly after completion of the study.

Coagulation assays

The aPTT and TCT were measured using commercially available reagents (Pathromtin, and thrombin reagent; Behringwerke AG, Marburg, Germany), in accordance with the manufacturer’s instructions; normal ranges, 28–40 s for aPTT and 12–20 s for TCT, respectively. Heptest was performed as described earlier [15]. FXa and Recalmix were from Heamachem (St. Louis, Missouri, USA); normal range, < 0.01 IU/ml. All clotting times were measured in duplicate on a KC-10 ball coagulometer (Amelung, Lemgo, Germany). The TFPI was measured in accordance with the protocol of Sandset et al. [16]; normal range, 0.7–1.2 /ml. Anti-FXa activities of Certoparin were measured with the chromogenic substrate S2222 and purified bovine FXa (Chromogenix, Essen, Germany); normal range, < 0.01 IU/ml for plasma and urine [17]. The activities of antithrombin were measured using the chromogenic substrate S 2238 method and purified thrombin (Chromogenix, Essen, Germany); normal range, < 0.02 IU/ml. The tests were performed according to standard laboratory procedures. The values are presented as mean and standard deviation.

Calculation of the pharmacodynamic parameters

As the plasma concentration of LMWH cannot be determined directly, anti-FXa activity is generally
accepted as a surrogate parameter for the plasma concentration. The following pharmacokinetic variables were derived directly from the measured anti-FXa activity: $C_{\text{max}}$, the maximal anti-FXa activity; $T_{\text{max}}$, the time point of the maximal anti-FXa activity; $T_{1/2}$, the elimination half-time; $C_l$, the total body clearance. The area under the activity-time curve before and 24 hrs after administration (time 0 to 24) (AUC$_{0-24}$) was determined by the trapezoidal rule, and $C_{\text{max}}$ and $T_{\text{max}}$ were taken directly from the measured data. AUC$_{0-8}$ and $T_{1/2}$ were extrapolated by mathematical fitting.

The sample size was estimated based on data from already published studies and the regulatory limits for bioequivalence. For the pharmacodynamic parameters, data from prior studies with other dosages were available. The coefficient of variation regarding AUC$_{0-24}$ was between 15 and 30% in these studies, with a total bioavailability of about 90%. At a coefficient of variation of 25%, it was expected that a sample size of 16 subjects included in the evaluation would allow a decision on bioequivalence. Under the assumption that at maximum 10% of the subjects had to be excluded from analysis, 18 subjects were enrolled in the study.

A test for equivalence was performed to investigate whether the AUC$_{0-24}$ after subcutaneous application was equivalent to that after intravenous administration. Limits of equivalence were set according to international standards as 100% (range, 80–125%). The null hypothesis was rejected by the method of two one-sided $t$ tests at a level of $\alpha = 5\%$, if the afore-mentioned confidence interval did not exceed these limits. The ratio of the AUC s.c./i.v. after administration of Certoparin was analysed on the basis of a multiplicative model after logarithmic transformation using an adequate model of variance analysis for a cross-over comparison. Data were analysed by means of the SAS system (version 6.12 for Windows NT 4, Cary Corp., North Carolina, USA) for general statistical analyses and modelling. The software WinNonlin (version 3.0; Pharsight Corp., Mountainview, California, USA) was used for calculating the pharmacokinetic/pharmacodynamic variables.

Safety
Safety and tolerability were evaluated from the frequency of adverse events and the number of laboratory values outside normal ranges. For laboratory values and changes of laboratory values, respectively, shift tables and simple statistical calculations (mean values, median, standard deviation, and range) were created. Noticeable values were marked in listings. Blood pressure and heart rate were analysed the same way.

Results
Eighteen healthy male volunteers were enrolled in the clinical, randomized cross-over trial and completed the study per protocol. Thus the safety population, intent to treat population and the per-protocol population were identical. Subjects’ mean age was 27.2 years [standard deviation (SD), 4.3; range, 22–36 years], mean height was 180.0 cm (SD, 7.5; range, 170.0–196.0 cm), and mean body weight was 72.9 kg (SD, 8.3; range, 60.0–88.0 kg).

Clinical and biological safety
There were a total number of four adverse events (AE) during the study period. Two subjects experienced burning pain at the site of s.c. injection. One subject had a 20 s episode of vasovagal syncope 2–3 min following intravenous administration, but also reported that he has experienced this kind of AE three times in the history following a venipuncture. One subject showed phlebitis at the site of the permanent venous canula after the intravenous administration of the compound. This reaction was treated with local drug therapy. There was no serious AE and no clinically significant effect on routine laboratory parameters or vital signs. In no case did treatment have to be discontinued.

Assessment of bioequivalence
The 90% confidence interval for data of the anti-FXa activity ranged from 93.30 to 110.02% (mean, 101.3%) and confirmed an equivalent availability for the s.c. as compared with the i.v. administration of Certoparin.

Anti-FXa activity
$C_{\text{max}}$ was $1.38 \pm 0.20$ IU/ml following i.v. and $0.61 \pm 0.13$ IU/ml following s.c. administration. $T_{\text{max}}$ ranged from 120 to 480 min (mean, 247 ± 87 min) after s.c. application. The results of the calculation of the other pharmacodynamic parameters are presented in Table 1. The activity time-courses of the anti-FXa activity are shown in Figure 1a.

Anti-FXa activity in urine
The cumulated anti-FXa units measured within a 24 h urine-sampling period are presented in Figure 2. The cumulated anti-FXa units of the 24 h urine were 4.1% following i.v. dosing and 3.6% following s.c. dosing.
The pharmacodynamic parameters obtained from the Heptest data (IU/ml) are presented in Table 1. C<sub>max</sub> was found to be three times higher following i.v. compared with s.c. administration. T<sub>max</sub> ranged from 141 to 192 min (95% of the lower and upper limit; mean, 167 min) for the s.c. application. The point estimator for the equivalence ratio of the AUC<sub>0±24</sub> was 90.2% (90% confidence interval, 84.7±96.1%). The activity time course is shown in Figure 1b.

Anti-activated factor II activity

All pharmacological parameters showed clear differences between the s.c. and i.v. administration in contrast to the anti-FXa activity. T<sub>max</sub> was 240 ± 68.3 min after s.c. dosing. C<sub>max</sub> was 0.782 and 0.126 IU/ml after i.v. and s.c. administration, respectively (Table 1). AUC values were higher for the i.v. dosing, resulting in an antilog of the differences in the AUCs of about 50% (mean, 53.78%; 90% confidence interval, 48.60–59.51%). The activity–time curves are shown in Figure 1c.

Tissue factor pathway inhibitor

The time-course of the release of TFPI is depicted in Figure 1d after i.v. and s.c. application. T<sub>max</sub> was 82 ± 43 min after s.c. dosing. C<sub>max</sub> was 2.9 ± 0.9 IU/ml following i.v. and 1.8 ± 0.9 IU/ml following s.c. administration (Table 1). The equivalence analysis was significant, as demonstrated by the antilog of the point estimator for the calculation of differences between AUC<sub>0–24</sub> s.c. minus i.v. with 103.1% (90% confidence interval, 96.3–110.4%).

Discussion

The present study demonstrated the bioequivalence of s.c and i.v. fixed high doses of a LMWH preparation on the anti-FXa activity. A complete equivalence of the s.c. administered LMWH Cetopar is also demonstrated on the Heptest assay and on the release of TFPI. The effect of s.c.-applied LMWH on thrombin inhibition was around 50% as compared with i.v. dosing. In addition, some of the pharmacodynamic results differed between the parameters requiring interpretation.

### Table 1. Pharmacodynamic parameters obtained from the anti-activated factor X (anti-FXa), anti-activated factor II (anti-FIIa), Heptest and tissue factor pathway inhibitor (TFPI) data after subcutaneous (s.c.) and intravenous (i.v.) administration of 8000 IU low-molecular-weight heparin (n = 18)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Anti-FXa</th>
<th>Anti-FIIa</th>
<th>Heptest</th>
<th>TFPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s.c.</td>
<td>246.66</td>
<td>87.043</td>
<td>240.00</td>
<td>68.256</td>
</tr>
<tr>
<td>i.v.</td>
<td>18.333</td>
<td>6.417</td>
<td>16.667</td>
<td>4.851</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (IU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s.c.</td>
<td>0.605</td>
<td>0.127</td>
<td>0.126</td>
<td>0.034</td>
</tr>
<tr>
<td>i.v.</td>
<td>1.381</td>
<td>0.202</td>
<td>0.782</td>
<td>0.153</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–24&lt;/sub&gt; (IU/ml h)</td>
<td>5.753</td>
<td>1.173</td>
<td>0.938</td>
<td>0.278</td>
</tr>
<tr>
<td>s.c.</td>
<td>5.588</td>
<td>0.866</td>
<td>1.931</td>
<td>0.338</td>
</tr>
<tr>
<td>i.v.</td>
<td>5.982</td>
<td>1.235</td>
<td>0.935</td>
<td>0.253</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s.c.</td>
<td>4.9</td>
<td>1.1</td>
<td>3.2</td>
<td>1.7</td>
</tr>
<tr>
<td>i.v.</td>
<td>6.2</td>
<td>2.7</td>
<td>1.153</td>
<td>0.402</td>
</tr>
<tr>
<td>Clearance (ml/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s.c.</td>
<td>1396</td>
<td>312</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>i.v.</td>
<td>1406</td>
<td>197</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

SD, standard deviation; AUC, area under the curve; na, not available.
The mean \( T_{\text{max}} \) of the anti-FXa activity after s.c. dosage of various LMWHs ranged from 3 to 4 h [7–10], except for reviparin (2–3 h) [18]. \( T_{\text{max}} \) of Certoparin was similar to those of most LMWHs after s.c. administration. The rate of appearance of maximal plasma antithrombin activity was somewhat longer, but is not yet clearly established [7–10, 19–21]. In the present study, \( T_{\text{max}} \) did not differ for anti-FXa, Heptest, and anti-thrombin assays after s.c. application of LMWH Certoparin. It may be speculated that the shorter saccharide chains are absorbed more rapidly after s.c. administration compared with the high molecular saccharides of the LMWH. The absorption of the low-molecular-weight saccharides is 100% and that of the high-

![Figure 1](image1.png)  
![Figure 2](image2.png)
molecular-weight saccharides 50%, as demonstrated by the differences of the anti-FXa and anti-activated factor II (anti-FIIa) results of our study. The complete absorption of the LMWH takes place also more rapidly as can seen from the same duration of $T_{\text{max}}$ of both heparins. This, however, may vary for different LMWHs [14,22,23,24,25]. It has been assumed, that the difference of the bioavailability calculated from the two methods could be due to the assumption that the low-molecular-weight fractions of the LMWH preparations are absorbed completely and the high-molecular-weight fractions to a lower extent from the s.c. tissue [23]. This would explain also the differences of the bioavailability of the LMWH on FXa and FIIa in the present study.

In contrast, the release of TFPI was more rapid after s.c. application of LMWH compared with the other parameters. TFPI is released by UFH as well as by LMWH. Therefore the molecular weight fractions of the LMWH preparation are unlikely to explain this phenomenon. TFPI is bound to lipoproteins, platelets and endothelium. It may be suggested that the pools of TFPI are immediately released by the low-molecular-weight saccharides of LMWH after s.c. administration. It has been reported that the TFPI pool is limited and may be exhausted. Thus, the high-molecular-weight saccharide chains of the LMWH that are absorbed more slowly do not find any TFPI for release.

The ratio of $C_{\text{max}}$ of the anti-FXa, Heptest and TFPI activity after i.v. compared with s.c. administration ranged from 2 to 3, and for the anti-FIIa activity was about 6. This is caused in part by absorption and elimination phenomena during the first 4 h after administration, and to some extent also by the complete bioavailability of LMWH towards anti-FXa activity and the 50% bioavailability towards anti-FIIa activity. The other pharmacodynamic characteristics of the parameters differed to a higher extent after i.v. and s.c. administration. The longer half-life of LMWHs has been reported by binding more tightly to antithrombin [24] and leukocytes [25], and by binding less to other plasma proteins [26] and endothelial cells [27], and to be metabolized less by macrophages [28] and the hepatic scavenger receptor [29].

The uptake was detected from an increased volume of distribution following i.v. administration and appeared to be restricted to saccharides between 3000 and 5000 Da [30]. It is assumed that several organs take up these molecular weight fractions and re-distribute them without relevant degradation [31]. This is also consistent with our findings of a larger volume of distribution calculated from the anti-FXa activity compared with the anti-FIIa activity.

In conclusion, the bioequivalence of a fixed dose of 8000 anti-FXa units of the LMWH Certoparin has been demonstrated following subcutaneous ad-
ministration in volunteers based on the plasma anti-FXa, Heptest and TFPI activity. Only small amounts of biologically active material were excreted into the urine. The pharmacodynamic studies have to evaluate these findings in patients with thromboembolism and other diseases following single and multiple administration at a dosage of 2 × 8000 anti-FXa IU.

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