Assessment of bioequivalence after subcutaneous and intramuscular administration of urinary gonadotrophins*

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The objective was to demonstrate bioequivalence between s.c. and i.m. administration of Humegon® (FSH/LH ratio 1:1) and Normegon® (FSH/LH ratio 3:1). In two randomized, single-centre, cross-over studies, 18 healthy volunteers on each formulation were assigned to one of the two administration sequences. Subjects were given single doses of one of the above gonadotrophins after endogenous gonadotrophin production had first been suppressed using high-dose oral contraceptive. Subsequently, rate (C max , t max ) and extent (AUC) of absorption of follicle stimulating hormone (FSH) and luteinizing hormone (LH) were determined for 14 days. For C max and AUC, analysis of variance (ANOVA) was performed on log-transformed data and for t max ANOVA was performed on ranks. Intramuscular and s.c. injections of Humegon were bioequivalent with respect to the main pharmacokinetic parameters, being AUC and C max of FSH absorption. Intramuscular and s.c. injections of Normegon were bioequivalent with respect to the AUC of FSH and not bioequivalent with respect to the C max of FSH. For t max of FSH as well as for most LH variables of both preparations, bioequivalence could not be be proven due to the high intra- and interindividual variability and/or concentrations being close to the detection limit. Thus, the main pharmacokinetic FSH variables after i.m. and s.c. administration of Humegon and Normegon were bioequivalent.

Key words: bioequivalence/FSH/human menopausal gonadotrophin/intramuscular/LH/subcutaneous

Introduction

Human gonadotrophin preparations currently available are natural hormones derived from female urine obtained either after menopause (human menopausal gonadotrophin; HMG) or during the first trimester of pregnancy (human chorionic gonadotrophin; HCG). The standard HMG preparations (Humegon®, Pergonal®) contain follicle stimulating hormone (FSH) and luteinizing hormone (LH) bioactivity in a ratio of approximately 1:1. The luteinizing activity of Humegon is standardized with HCG. Pergonal also contains HCG quantitatively and qualitatively indistinguishable from HCG in Humegon (Stokman et al., 1993). A new urinary HMG preparation, Normegon®, has been developed which is identical to Humegon, with the exception that the raw material is not standardized with HCG. Since the urine of postmenopausal women contains FSH and LH in a ratio of ~3:1, Normegon consequently also contains FSH and LH in a ratio of 3:1 (Duikers et al., 1993; Diedrich et al., 1994). Both preparations are intended for the treatment of female and male fertility disorders resulting from inadequate gonadotrophin stimulation of the gonads as well as for controlled ovarian hyperstimulation in medically assisted reproduction programmes.

Urinary gonadotrophins have been used for >30 years in the treatment of female and male infertility and most of them are indicated for i.m. use. As a result of this administration route and the possible requirements for injections during the weekend and holidays, practical problems may arise such as the necessity for daily visits to the clinic, and the almost continuous need for specialized assistance of (para-)medical staff. Consequently, the procedure may be inconvenient for both patients and hospital staff. Therefore, s.c. self-injection of gonadotrophin preparations may offer advantages for both the patients (and their partners) and the hospital in terms of convenience and workload.

Until recently, it was believed that urinary gonadotrophins could not be injected s.c., since the administration of these preparations with relatively high amounts of impurities via this route may induce undesirable local adverse reactions (Le Cotonnec et al., 1993). Although data have been presented indicative of an allergic potential of s.c. injected urinary gonadotrophins in a non-homologous animal model (Biffoni et al., 1994), clinical studies performed so far have indicated that the risk of allergic reactions is minimal and they support the safe administration of urinary gonadotrophins via the s.c. route (Saal et al., 1991a,b; Jones and Darne, 1993; Jones et al., 1994; Schmoutziguer et al., 1996). Thus, the bioequivalence issue of s.c. and i.m. administration of gonadotrophins is important in clinical decision making when choosing an administration route for these preparations which will remain available for an indeterminate period. The objective of this study was to investigate whether the i.m. and s.c. administration routes of two urinary gonadotrophin preparations are bioequivalent.

Materials and methods

In two single-centre, randomized, open-label, cross-over studies in pituitary suppressed women, the bioequivalence of s.c. and i.m.
Bioequivalence of s.c. and i.m. gonadotrophins

On day 22 of oral contraceptive treatment, a single dose of Humegon or Normegon was administered by means of either i.m. or s.c. injection, depending on the treatment allocation. On day 36, the procedure was repeated, but now in a two-way cross-over fashion as far as the route of administration was concerned. After each injection regular blood sampling followed for 14 days. All i.m. injections were administered in the upper lateral quadrant of the m. gluteus maximus, whereas the s.c. injections were administered in the frontal part of the thigh.

Before entering the study, the following assessments were made: medical, gynaecological and drug anamneses were obtained, physical and gynaecological examinations were performed, a pregnancy test was performed to exclude pregnancy, blood samples were obtained for measurement of FSH and LH immunoactivity as well as for routine blood biochemistry and haematology. With the exception of the pregnancy test and gonadotrophin determinations, the same assessments were performed at the post-treatment visit. Compliance to the daily intake of Lyndiol was checked in both studies by means of regular inquiry by the investigators and also by the assessment of FSH and LH serum concentrations on the day prior to Humegon or Normegon injection. An FSH concentration of <0.5 IU/l immunoactivity was considered to be indicative of full pituitary suppression.

In the Humegon and Normegon study groups blood samples for determination of FSH- and LH-immunoactivities were taken at the following times related to injection: –1, 1/2, 2, 3, 4, 6, 8, 10, 12, 16, 24, 30, 36, 48, 72, 96, 120, 168, 192, 240, 264, 312 and 336 h. After blood sampling, the serum was prepared and stored frozen until the assessments. FSH and LH immunoactivities were determined using a commercially available immunofluorometric assay using the time-resolved fluorimunnoassay technique (Delfia®; Wallac Oy, Turku, Finland). In both studies the following pharmacokinetic variables were assessed: peak serum concentration (C_{max}), peak serum time (t_{max}), and the area under the serum concentration versus time curve (AUC). For non-detectable concentrations a concentration of zero was assumed, and for missing values the area was calculated from the measurement preceding the missing value to the measurement following the missing value.

Comparison of the demographic data at screening was performed by means of a Wilcoxon rank-sum test on the two subject groups. The bioequivalence of the two administration routes was tested for the rate of absorption using C_{max} and t_{max}. Whereas for the extent of absorption bioequivalence was tested using AUC. For C_{max} and AUC an analysis of variance (ANOVA) was performed on the log-transformed values and the 90% confidence intervals (CI) were derived from the ratio of the means μ(s.c.)/μ(i.m.). For t_{max}, the ANOVA was performed on ranks and the 90% non-parametric CIs were determined using Walsh differences for the true difference between the means μ(s.c.) – μ(i.m.).

For determination of clinically relevant differences acceptance ranges were defined as follows: for the ratio of the means [μ(s.c.)/μ(i.m.)] of C_{max} and AUC, the range from 0.8–1.25 was used and for the true difference between the means [μ(s.c.) – μ(i.m.)] of t_{max}, ±20% of the i.m. mean was used. The formulations were declared bioequivalent with respect to a certain parameter if the 90% CI was fully within the acceptance range for that particular parameter, according to the recommendations of the International Harmonisation and Consensus DIA Meeting on bioavailability testing requirements and standards (Cartwright, 1991). For C_{max} and AUC if the 90% CI was within the acceptance range, then bioequivalence was proven; if the value 1 (0 for t_{max}) was within the 95% CI but the 90% CI was outside the acceptance range, then bioequivalence was not proven, since the outcome was inconclusive due to large intra- and interindividual variation; if the value 1 (0 for t_{max}) was outside the 95% CI

injection of Humegon or Normegon (N.V. Organon, Oss, The Netherlands) was investigated. Each study included 18 healthy volunteers and both studies were performed according to similar study protocols at the TNO Nutrition and Food Institute in Zeist (The Netherlands). This number of subjects is considered to have sufficient power to give information on the actual pharmacodynamic and pharmacokinetic response, based on prior experiences in studies with a similar design (Out et al., 1995). Assignment of subjects to the sequence of i.m. or s.c. injection of the preparations occurred with help of randomization lists. In order to reduce variability caused by endogenous release of FSH and LH, all women used during the study period the high-dose combined oral contraceptive Lyndiol® to suppress endogenous gonadotrophin production. A high-dose combined preparation was chosen, since it has been demonstrated that low-dose combined oral contraceptives generally do not ensure complete pituitary inhibition in all subjects (Dericks-Tan et al., 1976). All subjects gave their written informed consent to participate in the studies and the principles of the revised Declaration of Helsinki were implemented in the study. The studies were approved by the TNO Medical Ethics Committee and were performed according to Good Clinical Practice.

Inclusion criteria were: age between 18 and 39 years of age at the time of screening, use of combined oral contraceptives containing ≥30 µg ethinyl oestradiol per day during at least 3 months prior to the study, a history of normal cycles with a mean length of 24–35 days and an intra-individual variation of ±3 days before the use of oral contraceptives, a good physical and mental health, a body weight of 50–75 kg and between 80 and 130% of the ideal body weight (adapted from the Metropolitan Life Insurance Company tables), willing to give written informed consent. Exclusion criteria were: a history of, or current, endocrine abnormalities such as hyperprolactinaemia, polycystic ovary disease or absence of ovarian function, contraindications for the use of combined oral contraceptives or gonadotrophins, sitting diastolic blood pressure >90 mmHg and/or a systolic blood pressure >150 mmHg, chronic cardiovascular, hepatic, renal or pulmonary disease, a history (within 12 months) of or current abuse of alcohol or drugs, treatment with gonadotrophin preparations within a period of 6 months prior to screening, blood donation within 90 days prior to screening, concomitant use of medication (except for occasional analgesics), smoking >10 cigarettes per day, laboratory values indicative of physical illness, and use of investigational drugs within 3 months prior to screening.

Lyndiol (containing 50 µg ethinyl oestradiol and 2.5 mg lynestrenol per tablet; batches 93A04 and CP904072) is a high-dose oral contraceptive preparation. Starting on day 1 of the study, subjects were instructed to take one tablet per day for 50 consecutive days in order to achieve full pituitary suppression before the gonadotrophin injections (on days 22 and 36) and subsequently throughout the blood sampling period. Humegon (FSH/LH ratio 1:1; batch CP903153) was supplied as a freeze-dried, lyophilized powder in ampoules containing 75 IU of FSH and 75 IU of LH in-vivo bioactivity, as determined in the ovarian weight augmentation assay (Steelman and Pohley, 1953) and the seminal vesicle weight assay (Van Hell et al., 1964). For injection, four ampoules of Humegon (containing in total 300 IU FSH-bioactivity and 300 IU LH-bioactivity) were dissolved in 2 ml solvent. Normegon ([Org 31338] FSH/LH ratio 3:1, batch CP900033) was supplied as a freeze-dried, lyophilized powder in ampoules containing 75 IU of FSH and 25 IU LH in-vivo bioactivity, as determined in the ovarian weight augmentation assay (Steelman and Pohley, 1953) and the seminal vesicle weight assay (Van Hell et al., 1964). For injection, four ampoules of Normegon (containing in total 300 IU FSH-bioactivity and 100 IU LH-bioactivity) were dissolved in 2 ml solvent.
Table I. Bioequivalence testing (mean ± SD) of s.c. and i.m. Humegon injection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IM</th>
<th>SC</th>
<th>µSC/µIM (µSC-µM)</th>
<th>95% CI</th>
<th>90% CI</th>
<th>Outcome</th>
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<tr>
<td><strong>FSH variables</strong></td>
<td></td>
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<td></td>
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<tr>
<td>C\text{max} (IU/l)</td>
<td>3.92 ± 0.65</td>
<td>4.34 ± 0.77</td>
<td>1.10</td>
<td>1.02–1.20</td>
<td>1.03–1.18</td>
<td>Bioequivalent</td>
</tr>
<tr>
<td>t\text{max} (h)</td>
<td>25.8 ± 11.7</td>
<td>24.1 ± 10.4</td>
<td>–1.00</td>
<td>–9.99–6.02</td>
<td>–7.02–6.00</td>
<td>Bioequivalence not proven</td>
</tr>
<tr>
<td>AUC\text{0–336} (IU·h/l)</td>
<td>385.9 ± 79.6</td>
<td>413.1 ± 77.3</td>
<td>1.07</td>
<td>0.98–1.18</td>
<td>1.00–1.16</td>
<td>Bioequivalent</td>
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<tr>
<td><strong>LH variables</strong></td>
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<tr>
<td>C\text{max} (IU/l)</td>
<td>0.52 ± 0.42</td>
<td>0.49 ± 0.21</td>
<td>1.01</td>
<td>0.79–1.29</td>
<td>0.83–1.24</td>
<td>Bioequivalent</td>
</tr>
<tr>
<td>t\text{max} (h)</td>
<td>5.5 ± 3.3</td>
<td>9.7 ± 7.9</td>
<td>3.00</td>
<td>–0.05–7.45</td>
<td>0.00–6.02</td>
<td>Bioequivalence not proven</td>
</tr>
<tr>
<td>AUC\text{0–336} (IU·h/l)</td>
<td>8.8 ± 9.0</td>
<td>11.0 ± 12.4</td>
<td>1.52</td>
<td>0.95–2.44</td>
<td>1.03–2.25</td>
<td>Bioequivalence not proven</td>
</tr>
</tbody>
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and also the 90% CI was outside the acceptance range, then non-bioequivalence was proven.

Statistical calculations were performed using SAS version 6.06 under VAX/VMS operating system V5.3.

Results

All subjects in the Humegon study group completed the studies. Of the 18 women randomized in the Normegon study, 15 completed it successfully whereas three subjects discontinued for reasons unrelated to treatment: one woman was excluded after erroneous injection of Humegon instead of Normegon, one subject was discontinued because of the occurrence of an intercurrent bacterial infection requiring antibiotic treatment, and one woman withdrew from the study shortly after starting oral contraceptive treatment. In the Humegon study, the mean age of the subjects was 21.4 ± 2.2 years (range 18–26), the mean height was 175.4 ± 6.3 cm (range 158–184) and the mean body weight was 63.9 ± 6.1 kg (range 53–74). In the Normegon study the corresponding figures were 21.6 ± 2.1 years (range 19–26), 175.1 ± 7.4 cm (range 156–186) and 65.9 ± 5.7 kg (range 54–75). Comparison of the average age, height and body weight of the different administration sequences within each study using a Wilcoxon rank-sum test revealed no statistically significant between-sequence differences.

The mean values of the pharmacokinetic parameters obtained during bioequivalence testing of the i.m. and s.c. administration routes of Humegon are presented in Table I. The plots of the mean FSH and LH serum levels per treatment group over time are presented in Figures 1 and 2 respectively. There were neither statistically nor clinically significant differences between the mean AUC\text{0–336} of FSH for s.c. and i.m. Humegon injection and consequently bioequivalence was proven. For C\text{max} of FSH, although the difference between i.m. and s.c. injection was statistically significant, the 90% CI was still within the acceptance range. Therefore, the difference was clinically not significant and bioequivalence was still proven. For t\text{max} of FSH, although there were no statistically significant between-group differences, there was a large intra- and interindividual variability with the 90% CI outside the acceptance range. Consequently, bioequivalence could not be proven. With respect to FSH, for both C\text{max} and AUC\text{0–336} a period effect was found. Subjects receiving Humegon on day 36 had been exposed to the oral contraceptive preparation for 14 days longer than subjects receiving Humegon on day 22. This may have resulted in a stronger suppression of endogenous FSH release during the second period and consequently to lower residual serum concentrations.

The mean AUC\text{0–336} of LH seemed to be larger after s.c. than after i.m. Humegon injection. Although the difference between the two administration routes was not statistically significant, bioequivalence could not be proven (90% CI outside the acceptance range). The C\text{max} was similar for i.m. and s.c. injection, since the between-group differences were neither statistically nor clinically significant. Therefore, for
Bioequivalence of s.c. and i.m. gonadotrophins

<table>
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<th>Parameter</th>
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<td></td>
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<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (IU/l)</td>
<td>3.69 ± 0.79</td>
<td>4.42 ± 1.10</td>
<td>1.20</td>
<td>1.02–1.43</td>
<td>1.05–1.38</td>
<td>Non-bioequivalent</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>25.6 ± 15.2</td>
<td>21.3 ± 10.3</td>
<td>(–4.62)</td>
<td>–13.25–5.97</td>
<td>–12.99–4.00</td>
<td>Bioequivalence not proven</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–336&lt;/sub&gt; (IU·h/l)</td>
<td>369.7 ± 68.9</td>
<td>404.5 ± 62.2</td>
<td>1.11</td>
<td>1.00–1.23</td>
<td>1.02–1.20</td>
<td>Bioequivalent</td>
</tr>
<tr>
<td><strong>LH variables</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (IU/l)</td>
<td>0.33 ± 0.17</td>
<td>0.41 ± 0.18</td>
<td>1.17</td>
<td>0.99–1.37</td>
<td>1.02–1.33</td>
<td>Bioequivalence not proven</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>5.9 ± 4.7</td>
<td>9.2 ± 7.0</td>
<td>(3.02)</td>
<td>–1.25–8.51</td>
<td>–0.50–7.00</td>
<td>Bioequivalence not proven</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–336&lt;/sub&gt; (IU·h/l)</td>
<td>4.9 ± 6.5</td>
<td>6.6 ± 6.1</td>
<td>1.75</td>
<td>0.78–3.90</td>
<td>0.91–3.36</td>
<td>Bioequivalence not proven</td>
</tr>
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</table>

Figure 3. Follicle stimulating hormone (FSH) serum concentrations versus time after s.c. or i.m. injection with Normegon.

Figure 4. Luteinizing hormone (LH) serum concentrations versus time after s.c. or i.m. injection with Normegon.

this parameter bioequivalence was proven. With respect to t<sub>max</sub>, absorption seemed to be faster after i.m. than after s.c. injection. Although the between-group difference was not statistically significant, bioequivalence could not be proven (90% CI outside the acceptance range). Similar to the findings obtained for FSH, with LH a period effect was found for C<sub>max</sub> and AUC<sub>0–336</sub>, resulting in a stronger suppression of endogenous LH release during the second period.

The mean values of the pharmacokinetic parameters used during bioequivalence testing of i.m. and s.c. injection of Normegon are presented in Table II. The plots of the mean FSH and LH serum concentrations per treatment group over time are presented in Figures 3 and 4 respectively. For the AUC<sub>0–336</sub> of FSH, although the difference between i.m. and s.c. injection was statistically significant, the 90% CI was still within the acceptance range. Therefore, the difference was clinically not significant and bioequivalence was proven. For the C<sub>max</sub>, the two administration routes of Normegon revealed statistically significantly different peak plasma concentrations and since the 90% CI was also outside the acceptance range, it was concluded that the two administration routes were not bioequivalent for C<sub>max</sub>. For the t<sub>max</sub>, bioequivalence could not be proven. A period effect was found for the AUC<sub>0–336</sub> of FSH.

Bioequivalence with respect to LH could not be proven for any of the investigated pharmacokinetic parameters (90% confidence intervals outside the acceptance range).

Common side effects attributable to the oral contraceptive preparation were reported in most of the subjects. Although both gonadotrophin administration routes caused some irritation and/or pain at the injection site in most of the subjects, starting several hours after injection and lasting up to several days, the i.m. as well as the s.c. injections with Humegon and Normegon were well tolerated.

**Discussion**

From the current study it appeared that, in terms of the extent of FSH absorption, as measured by the AUC, the s.c. administration route for Humegon is bioequivalent with the i.m. route. In addition, bioequivalence could also be proven for the rate variable of FSH absorption C<sub>max</sub>. With respect to LH variables, bioequivalence of the two administration routes could be proven for C<sub>max</sub>. For t<sub>max</sub> of FSH and LH, bioequivalence could not be proven due to the large intra- and interindividual variability. Non-bioequivalence was not proven for a particular variable after i.m. and s.c. Humegon injection. Since for follicular development FSH pharmacokinetics are much more important than those of LH, proof of LH bioequivalence is of little relevance for the efficacy of Humegon. Although a period effect (due to a stronger suppression of endogenous FSH with longer duration of administration of the oral contraceptive preparation) for both C<sub>max</sub> and AUC of FSH and LH was demonstrated, this was not considered to have reduced the usefulness of the statistical model since the study was per-
formed in a cross-over fashion. The finding of bioequivalence of the s.c. and i.m. administration routes for the main pharmacokinetic variables of Humegon is supported by a recent retrospective analysis of more than 1200 in-vitro fertilization (IVF)/embryo transfer cycles with s.c. injection of Humegon, in which a clinical pregnancy rate was obtained that was comparable with that from earlier studies in which Humegon was administered i.m. (Schmouhtzigue, et al., 1996).

From the current study it appeared that in terms of the AUC, the s.c. administration route of Normegon is bioequivalent with the i.m. route. With respect to LH, bioequivalence of the two administration routes could not be proven for C max, AUC and t max due to the large intra- and interindividual variability. This variability may have resulted from some residual endogenous LH production as well as to the fact that most LH values were close to or below the detection limit. Similar to Humegon, since for follicular development FSH pharmacokinetics are much more important than those of LH, proof of LH bioequivalence is of little relevance for the efficacy of the preparation. Although a period effect for the AUC of FSH was demonstrated, again this was not considered to have reduced the usefulness of the statistical model.

The results obtained with Humegon and Normegon in the current study with respect to the bioavailability of the s.c. and the i.m. administration routes are in accordance with a similar bioequivalence study with an FSH preparation (Le Cotonne, et al., 1993). On the other hand, they deviate in part from a recently published bioequivalence study with another urinary HMG preparation with a FSH/LH ratio of 1:1 (Pergonal), in which s.c. and i.m. injection were compared. In that study, although there were no differences between the administration routes with respect to C max of FSH, the t max of FSH was significantly higher after s.c. injection (Dobbs et al., 1994). Whether these differences between the administration routes bear any clinical relevance should be further investigated.

In conclusion, from this study it appears that the s.c. and i.m. administration routes of the urinary gonadotrophin preparations Humegon and Normegon are bioequivalent with respect to the main pharmacokinetic variables. This is supported by data from clinical studies which have indicated that s.c. and i.m. administered gonadotrophins may be equally effective. Definitive proof of absence of a clinically relevant difference between s.c. and i.m. injection of urinary gonadotrophin preparations should come from well-controlled, prospective clinical efficacy studies which are currently being performed.

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References


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