Single dose pharmacokinetics and effects on follicular growth and serum hormones of a long-acting recombinant FSH preparation (FSH-CTP) in healthy pituitary-suppressed females

Ingrid J.M. Duijkers1,3, Christine Klipping1, Peter J. Boerrigter2, Christel S.M. Machielsen2, Joris J. de Bie2 and Gerrit Voortman2

1Dinox Medical Investigations, Groenewoudseweg 317, 6524 TX, Nijmegen and 2N.V. Organon, Molenstraat 110, P.O.Box 20, 5340 BH, Oss, The Netherlands

3To whom correspondence should be addressed. E-mail: dinox@molyvos.net

BACKGROUND: A long-acting FSH preparation has been developed by site-directed mutagenesis and gene transfer techniques. METHODS: In this open-label trial, we investigated the pharmacokinetic and pharmacodynamic properties of FSH-CTP (corifollitropin alpha, Org 36286) in healthy female volunteers. Twenty-four subjects were treated with a high-dose oral contraceptive (OC) to suppress pituitary function. A single dose of 15, 30 or 60 µg FSH-CTP was injected (s.c., eight subjects per dose group) and seven of these 24 subjects were subsequently treated with a single dose of 120 µg. RESULTS: Maximum serum FSH-CTP concentrations (0.42, 0.66, 1.49 and 3.27 ng/ml after administration of 15, 30, 60 and 120 µg Org 36286 respectively) were reached between 36 and 48 h after injection and t 1/2 varied between 60 and 75 h. Dose proportionality was shown across the studied dose range, whereas t max and t 1/2 were dose independent. In most subjects follicular growth was observed; the number and maximum diameter of the follicles increased with the dose. Follicles with a diameter ≥8.0 mm were observed only in the 60 and 120 µg dose groups, diameters between 12.0 and 15.9 mm occurred only in the 120 µg group. Serum LH and 17β-oestradiol levels remained low due to profound pituitary suppression whereas inhibin-B levels increased with dose. Maximum mean inhibin-B levels were 30.4, 322.7 and 1059.3 pg/ml in the 30, 60 and 120 µg dose group respectively. The preparation was safe and well tolerated, and no FSH-CTP antibody formation was observed. CONCLUSIONS: The pharmacokinetics of FSH-CTP were shown to be proportional with the dose. The elimination half-life was approximately two times longer than that of rFSH. A single dose of FSH-CTP was shown to be safe and able to induce multiple follicular growth accompanied by a dose-dependent rise in serum inhibin-B concentrations.

Key words: FSH-CTP/infertility/long-acting/pharmacodynamics/pharmacokinetics

Introduction

FSH belongs, together with LH, HCG and thyroid stimulating hormone (TSH), to a family of glycoproteins that are heterodimers of two subunits, the α- and β-subunit. The α-subunit is identical for these hormones, the β-subunit is different and determines the biological specificity of each hormone. The β-subunits of LH and HCG are very similar except for a carboxy-terminal peptide extension of the HCG β-subunit. This carboxy-terminal peptide is crucial for the long terminal half-life of HCG (24 h) as compared with that of LH (2 h) (Matzuk et al., 1990; Speroff et al., 1994).

At present, recombinant human FSH (rFSH) preparations are widely used for infertility treatment. rFSH is produced by a Chinese hamster ovary (CHO) cell line transfected with the genes encoding for the two subunits of FSH. rFSH preparations have structural, biochemical and biological characteristics similar to those of urinary FSH preparations (Hård et al., 1990; Mannaerts et al., 1991; De Leeuw et al., 1996; Olijve et al., 1996). Like urinary preparations, rFSH preparations have to be administered by daily injections to achieve steady state FSH levels (Schoot et al., 1994).

A long-acting FSH preparation has been developed by site-directed mutagenesis and gene transfer techniques. This resulted in recombinant FSH consisting of an α-subunit identical to that of FSH, whereas the β-subunit is a hybrid composed of the FSH β-subunit and the carboxy-terminal peptide (CTP) of the HCG β-subunit (Fares et al., 1992). Because of this CTP component, which contains four O-linked oligosaccharides (Boime and Ben-Menahem, 1999),
this FSH-CTP preparation was expected to have a prolonged half-life compared to rFSH. Animal studies have shown a prolonged terminal elimination half-life and increased in-vivo bioactivity of FSH-CTP compared with rFSH (Fares et al., 1992; LaPolt et al., 1992). The first clinical study in hypogonadotrophic hypogonadal men showed relatively slow absorption of FSH-CTP and a half-life which was approximately two to three times longer than that of rFSH (Bouloux et al., 2001).

The aim of the present study was to determine pharmacokinetic parameters and to investigate the pharmacodynamics, i.e. follicular growth and serum hormone levels, of a single s.c. dose of FSH-CTP in healthy pituitary-suppressed female volunteers. Serum inhibin-B and 17β-oestradiol (E2) levels were determined as hormonal parameters for follicular growth.

Materials and methods

Subjects
All subjects who participated in this trial gave their written informed consent and the study was approved by an independent ethical committee. Main inclusion criteria were: age between 18 and 38 years, body mass index between 18 and 29 kg/m², and good health. The most important exclusion criteria were: hypertension, contraindications for the use of oral contraceptives or gonadotrophins, history of endocrine abnormalities such as hyperprolactinaemia, polycystic ovarian syndrome or ovarian dysfunction, ovarian cysts or enlarged ovaries, history of ovarian surgery, smoking >10 cigarettes per day, history of alcohol or drug abuse. Before inclusion into the study, all subjects underwent a general physical and gynaecological examination, including transvaginal ultrasonography and cervical smear. Haematological and clinical chemical blood parameters were determined and urinalysis was performed.

In total, 24 healthy female volunteers participated in the study. The first group of eight subjects was treated with 30 µg, the second group with 15 µg, and the third group with 60 µg FSH-CTP. Since the follicular growth in the highest dose group was less pronounced than in a previous study with a similar design with rFSH, which was administered daily for 7 days (Voortman et al., 2000), it was decided to add a fourth group to be treated with 120 µg FSH-CTP. The fourth group consisted of subjects who had already participated in one of the first three dosing groups (eight subjects in total, four subjects already exposed to 30 µg, two subjects already exposed to 15 µg, two subjects already exposed to 60 µg). The time between two injections with FSH-CTP was ≈3 months. In total, only seven subjects were exposed to 120 µg. One subject discontinued before administration because of an ovarian cyst during pre-treatment.

Treatment
After inclusion of a subject in the study, she was asked to discontinue the use of her own oral contraceptive during 1 week. After the pill-free period, she started the daily intake of a combined oral contraceptive (Lyndiol®; containing 50 µg ethinyl estradiol and 2.5 mg lynestrenol) for a period of 6 weeks, to suppress endogenous gonadotrophin secretion. On the 21st day of pill intake, the subjects were admitted to the study centre. The next day, between 08:00 and 09:10, a single dose of FSH-CTP (corifollitropin alpha, Org 36286) was injected s.c. into the abdominal wall (treatment day 1). The subjects remained in the study centre until 36 h after administration. Daily pill intake was continued for 21 days after FSH-CTP administration.

The FSH-CTP preparation was supplied as a solution for injection in vials containing 15 µg FSH-CTP in 0.5 ml solution each. The lower doses were administered by single injection, the 120 µg dose was administered by two injections of 2 ml solution.

Measurements
Transvaginal ultrasonography was performed on the 15th day of contraceptive pill intake, on the day before FSH-CTP administration, on treatment day 2, thereafter daily until day 7, then every other day until day 21. Ultrasonography was performed again at final examination. Ultrasonography was performed using an Eccocese device (Toshiba) with a 6 MHz vaginal probe. The mean diameter of bidirectional measurement of each follicle and the number of follicles with a mean diameter ≥5 mm was assessed at each visit.

Blood samples for determination of serum FSH-CTP concentrations were taken pre-dose and 2, 4, 6, 8, 12, 16, 24, 30, 36, 40, 48, 54, 60, 72, 96, 120 and 144 h after administration of the preparation, then every other day until treatment day 15. Serum LH, inhibin-B and E2 concentrations were determined pre-dose, then every 24 h until day 7, subsequently every other day until day 21, and at the final examination 2 weeks after discontinuing the contraceptive pill. Pre-dose and at the final examination, blood samples were taken for determination of antibodies against FSH-CTP and CHO-derived proteins.

Blood pressure, heart rate and body weight were measured on the 15th day of Lyndiol intake, on the day before FSH-CTP administration, and on treatment days 5 and 9. Haematological and clinical chemical blood parameters and urinalysis were checked on the 15th day of Lyndiol and on treatment day 5 and 9. On the day before FSH-CTP administration, a urine drug screen and pregnancy test were done. Two weeks after discontinuing Lyndiol, a general physical and gynaecological examination, haematological and clinical chemical blood control, urinalysis and pregnancy test were performed.

Assays
FSH-CTP serum levels and presence of antibodies directed against FSH-CTP or CHO-derived proteins were assayed as previously described (Bouloux et al., 2001). In short, FSH-CTP levels were determined using an enzyme immunoassay [EIA; coefficient of variation (CV) of quality controls did not exceed 20%, lower limit of quantification (LLOQ) 0.079 ng/ml]. Presence of antibodies directed against CHO-derived proteins and FSH-CTP was assessed by a qualitative EIA and radioimmunoassay respectively. Detection of antibodies directed against FSH-CTP was based on the formation of an immune complex between the specific antibody and [125I]Org 36286.

Inhibin-B in human serum was denatured (using sodium dodecyl sulphate at 100°C), oxygenated (hydrogen peroxide), and subsequently analysed using an adapted enzyme immunoassay (Robertson et al., 1996). The LLOQ of this assay was 15.6 pg/ml and the CV of quality controls did not exceed 20%. LH and E2 serum levels were determined by the ABL assay (ABL, Assen, The Netherlands) using a time-resolved fluoroimmunoassay (Autodelphia®, Delfia; Wallac Oy, Turku, Finland). The LLOQ of E2 levels in serum was 13.6 pg/ml, LLOQ for LH levels was 0.6 IU/l. The CV of quality controls did not exceed 5% for both E2 and LH assays.
Pharmacokinetic evaluation

The peak FSH-CTP concentration (C\textsubscript{max}) and the time of its occurrence (t\textsubscript{max}) were taken from the measured serum concentration data; the dose-normalized C\textsubscript{max} (dn-C\textsubscript{max}) was calculated as C\textsubscript{max}/dose. The terminal elimination rate constant (λ\textsubscript{z}) and elimination half-life (t\textsubscript{1/2}) were calculated from the log-linear phase of the concentration–time curve by linear regression. Concentrations lower than the LLOQ in the elimination phase were ignored.

The area under the FSH-CTP serum concentration curve (AUC) from zero to t\textsubscript{last} (AUC\textsubscript{0–t\textsubscript{last}}) was calculated by means of the linear trapezoidal rule, where t\textsubscript{last} represents the last time point with a measurable concentration within a subject. The AUC from zero to infinity (AUC\textsubscript{0–∞}) was calculated as AUC\textsubscript{0–t\textsubscript{last}} + AUC\textsubscript{t\textsubscript{last}–∞}. In this formula, AUC\textsubscript{t\textsubscript{last}–∞} = C\textsubscript{t\textsubscript{last}}/λ\textsubscript{z} where C\textsubscript{t\textsubscript{last}} was the fitted concentration at time t\textsubscript{last} using the regression line from which λ\textsubscript{z} was calculated. The dose-normalized AUC\textsubscript{0–∞} (dn-AUC\textsubscript{0–∞}) was calculated as AUC\textsubscript{0–∞}/dose. The true total serum clearance (CL) after single-dose administration equals f*dose/AUC\textsubscript{0–∞} where f is the fractional absolute bioavailability of the s.c. preparation. Since f cannot be calculated from s.c. data alone, CL/f = dose/AUC\textsubscript{0–∞} was calculated and denoted as ‘apparent clearance’ (CL\textsubscript{app}).

Statistics

Descriptive statistics for the serum concentrations were only calculated if at least two-thirds of the concentration values by point time were ≥LLOQ. For calculation and plotting of the mean concentration–time curve, concentrations indicated as below the LLOQ were replaced by 0.5*LLOQ. Ignoring the values here would overestimate the means. This approach has no impact on pharmacokinetic parameter calculations. To test whether the pharmacokinetics after four different dose levels were dose-proportional, a one-way analysis of variance (ANOVA) was performed on the loge-transforms of the dn-AUC\textsubscript{0–∞} and dn-C\textsubscript{max}. Conclusions with respect to dose proportionality were based on these parameters only. Using the same one-way ANOVA, t\textsubscript{1/2} was tested for dose independence. For t\textsubscript{max}, the non-parametric Kruskal–Wallis test was performed to test for dose independence. Effects were considered statistically significant if P ≤ 0.05.

Results

Subject characteristics

The mean age, body weight and body mass index, were similar in all four different treatment groups and are presented in Table I.

Pharmacokinetic parameters

In three pre-dose samples, one in the 30 µg, one in the 60 µg and one in the 120 µg group, measurable FSH-CTP levels were found. The samples in the 30 and the 120 µg group were from the same subject, who was treated twice with an interval of 3.5 months. These subjects also had higher FSH-CTP levels at the end of the sampling period than the other subjects in the dose group. The results from these subjects were therefore excluded from the pharmacokinetic analysis, but not from the pharmacodynamic analysis. This phenomenon was also observed in previous study in hypogonadotrophic hypogonadal men and is most likely caused by non-specific serum protein interference (Bouloux et al., 2001).

One subject in the 15 µg group had relatively low FSH-CTP levels compared with the other subjects in this dose group. For this subject, the extrapolation from AUC\textsubscript{t\textsubscript{last}} to AUC\textsubscript{0–∞} was extremely high, therefore the pharmacokinetic parameters (dn-)AUC\textsubscript{0–∞} and CL\textsubscript{app} of the subject were not included in the analysis.

Serum FSH-CTP concentrations in the different dose groups are shown in Figure 1. A summary of pharmacokinetic parameters is given in Table II.
Table II. Pharmacokinetic parameters after single administration of 15, 30, 60 and 120 µg FSH-CTP during Lyndiol treatment

<table>
<thead>
<tr>
<th>Dose (µg)</th>
<th>t max (h)</th>
<th>C max (ng/ml/µg)</th>
<th>dn-C max (h)</th>
<th>t1/2 (ng*h/ml)</th>
<th>AUC0 (ng*h/ml/µg)</th>
<th>dn-AUC0 (ng*h/ml/µg)</th>
<th>CL app (l/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 µg</td>
<td>36</td>
<td>0.416</td>
<td>0.0278</td>
<td>68.4</td>
<td>61.7</td>
<td>4.11</td>
<td>0.243</td>
</tr>
<tr>
<td>30 µg</td>
<td>48</td>
<td>0.661</td>
<td>0.0220</td>
<td>59.5</td>
<td>80.1</td>
<td>2.67</td>
<td>0.375</td>
</tr>
<tr>
<td>60 µg</td>
<td>36</td>
<td>1.49</td>
<td>0.0249</td>
<td>64.8</td>
<td>187</td>
<td>3.11</td>
<td>0.321</td>
</tr>
<tr>
<td>120 µg</td>
<td>36</td>
<td>3.27</td>
<td>0.0272</td>
<td>74.5</td>
<td>385</td>
<td>3.21</td>
<td>0.311</td>
</tr>
</tbody>
</table>

For (dn-)AUC0 and CL app: n = 7.
Values are geometric mean, CV (%) and range. For t max: median (min – max) is presented.
For definitions, see Materials and methods.

For all doses tested, the median time to reach maximal serum concentrations (t max) appeared to be comparable (ranging from 36 h in the 15, 60 and 120 µg groups to 48 h after administration of 30 µg). The calculated elimination half-lives (t 1/2) ranged from 60 (30 µg group) to 75 h (120 µg group). Both t 1/2 and t max appeared to be dose independent within the dose range tested.

The maximal serum concentration of Org 36286 increased with the doses injected. Maximum serum FSH-CTP concentrations were 0.42, 0.66, 1.49 and 3.27 ng/ml after administration of 15, 30, 60 or 120 µg Org 36286 respectively. When statistical analysis was performed, no statistically significant differences between doses were found for any of the (dose-normalized) pharmacokinetic parameters.

Follicular growth
The results from the ultrasound measurements for subjects treated with 60 or 120 µg are depicted in Figure 2. The mean numbers of follicles per subject on each trial day are shown for the different dose groups. In two subjects in the 15 µg group and two subjects in the 30 µg group, all follicles remained <5 mm. Follicles with a diameter ≥8 mm were only observed in the 60 and 120 µg groups. Follicles between 12.0 and 15.9 mm were present in the 120 µg group only, whereas in none of the four treatment groups follicles ≥16 mm were seen.

The median value (range) of the maximum number of follicles with a diameter ≥5.0 mm was 1 (0–4), 1 (0–8), 15 (6–31) and 27 (4–47) follicles, in the 15, 30, 60 and 120 µg group respectively. The maximum number of follicles (≥5.0 mm) was observed on treatment day 6, 5, 7 and 9 (median) in the 15, 30, 60 and 120 µg group respectively.

Serum hormone levels
In almost all samples, LH concentrations were below the LLOQ, indicating adequate pituitary suppression by Lyndiol. However, in three subjects (one in the 15 µg, one in the 30 µg and one in the 60 µg group) LH levels were well above the LLOQ for several days after FSH-CTP administration, indicating insufficient suppression of endogenous gonadotrophins by Lyndiol. The results from these three subjects (ultra-
Sound and hormone measurements) were therefore excluded from the pharmacodynamic analysis but not from the pharmacokinetic analysis.

Serum E2 concentrations were close to the LLOQ, (13.6 pg/ml) throughout the treatment period. The maximum mean E2 concentration (24.7 pg/ml) was measured on treatment day 7, in the 120 μg group. The three subjects who were excluded from the pharmacodynamic analysis because of elevated LH levels had maximum E2 concentrations varying between 33.1 and 58.0 pg/ml.

Mean serum inhibin-B concentrations in the different dose groups are shown in Figure 3. On day 1, before FSH-CTP administration, inhibin-B concentrations were below the LLOQ in most samples. On day 2, mean serum inhibin-B concentrations were not calculated. Maximum mean serum inhibin-B levels were reached on day 3 in the 30 μg group (30.4 pg/ml), on day 4 in the 60 μg group (322.7 pg/ml), and on day 6 in the 120 μg group (1059.3 pg/ml). Thereafter, a gradual decrease of inhibin-B concentrations was observed.

**Safety**

No antibodies against FSH-CTP or CHO-derived proteins were detected. The FSH-CTP preparation was well tolerated. No serious adverse events (SAE) were observed and none of the subjects discontinued due to adverse events (AE). There were no clinically relevant adverse events and no relevant changes in laboratory parameters.

**Discussion**

The results from this first study with FSH-CTP in women of reproductive age showed that a single dose of FSH-CTP appeared to be safe and induced multiple follicular growth accompanied by a dose-dependent rise in serum inhibin-B concentrations. Furthermore, pharmacokinetic analysis showed that the absorption and elimination process of FSH-CTP was slow as compared with rFSH.

In the present study, comparing s.c. administration of 15, 30, 60 and 120 μg FSH-CTP in female subjects, maximum serum FSH-CTP concentrations were reached between 36 and 48 h (mean) after injection. The mean elimination half-life varied from 60 to 75 h. This finding was also observed in a previous study including 13 hypogonadotropic hypogonadal male subjects who received four single s.c. injections of 15 μg FSH-CTP (Bouloux et al., 2001). In this trial in men, maximum serum FSH-CTP concentrations were reached on average (± SD) after 46 ± 18 h and the average half-life was 95 ± 26 h. Half-life and t_max are much lower when rFSH is used instead of FSH-CTP. In a study in which a single dose of 300 IU rFSH (Puregon) was administered s.c. in 13 women during Lyndiol suppression (Mannaerts et al. 1996), maximum FSH concentrations were already reached after 17 h. After s.c. administration of 75, 150 or 225 IU Puregon daily for 7 days in females during Lyndiol suppression, the t_max varied between 7.5 and 8.5 h, and the terminal half-life between 34.8 and 36.2 h (Voortman et al., 2000). In agreement herewith, a single dose of 150 IU Gonal-F in pituitary-suppressed women resulted in a t_max of 16 h and a t1/2 of 37 h, whereas after 7 days of rFSH (150 IU) t_max and t1/2 were 8 and 24 h respectively (Le Cotonnec et al., 1994). We therefore conclude that the time to reach maximum serum concentrations after single s.c. administration in women is two to three times longer for FSH-CTP than for rFSH and the elimination half-life of FSH-CTP is approximately twice that of rFSH. Different mechanisms could account for the longer time to reach maximum serum concentrations of FSH-CTP. First, the FSH-CTP molecule is larger than the rFSH molecule, which could result in slower absorption. Furthermore, the elimination of FSH-CTP is slower than that of rFSH. After s.c. administration of a drug, the absorption process is still ongoing when the elimination process has already started. If the elimination of a drug is slower, the point at which the elimination process exceeds the absorption process (the point at which maximum concentrations are measured) will be at a later time point.

Transvaginal ultrasonography results showed that single FSH-CTP administration induced follicular growth in almost all subjects. The number and diameter of the follicles increased with the FSH-CTP dose. No follicles >8.0 mm were observed after single administration of 15 and 30 μg FSH-CTP. The mean number of follicles >8.0 mm on the day of maximum stimulation in the 60 and 120 μg groups was 1 and 9 respectively. The maximum diameter of follicles in the 60 μg group was between 8.0 and 9.9 mm and between 14.0 and 15.9 mm in the 120 μg group. In comparison, after daily s.c. administration of 75, 150, or 225 IU of rFSH (Puregon) for 7 days to female volunteers during Lyndiol treatment, the mean number of follicles >8 mm on the day of maximum stimulation was 2, 14 and
14 respectively (Voortman et al., 2000). In the 150 and 225 IU groups, follicles ≥16 mm were seen (Voortman et al., 2000). In contrast with the rFSH study, follicles with a diameter between 5 and 8 mm were also recorded in the present study. In the higher dose groups, a large cohort of follicles in this size class appeared to be recruited. The treatment day on which the maximum number of follicles was observed was comparable in the two studies: between days 7 and 9 in the rFSH study, and between days 5 and 9 in the present FSH-CTP study. When comparing the ultrasonography results of this study with results from previous work (Voortman et al., 2000), the effect of a single administration of 120 µg FSH-CTP on follicular growth appears to be slightly reduced compared with 7 days administration of 150 or 225 IU rFSH. This implies that, to obtain an effect similar to that of seven daily rFSH injections, the dose of FSH-CTP should be further increased. Thus, FSH levels would remain above the threshold level for follicular stimulation during a longer time period, and probably weekly administration would be sufficient.

If FSH-CTP is administered once weekly and has an effect similar to that of multiple daily rFSH injections, this will obviously reduce the discomfort of patients undergoing infertility treatment since fewer injections will be required. Especially in the initial phase of ovarian stimulation, a single injection of FSH-CTP could replace daily gonadotrophin injections. However, further clinical studies are needed to search for optimal treatment regimes.

In the current study, we also measured several serum hormone levels upon administration of FSH-CTP. Serum levels of inhibin-B, which is produced by granulosa cells and is an early marker of follicular growth (Groome et al., 1996), increased dose-dependently after FSH-CTP administration. Inhibin-B levels declined a few days before follicular growth was arrested, which is in agreement with results of an earlier study on the effect of 7 days rFSH administration (Porchet et al., 1994). In our trial, serum LH concentrations were lower than observed during GnRH agonist administration and comparable with those of hypogonadotropic hypogonadal women (Schoot et al., 1992; Mannaerts et al., 1993; Devroey et al., 1994).

Apparent LH levels, as observed in this trial, were too low for adequate E2 production since, even though E2 is a marker of follicular growth, E2 levels were also low. Only in the highest dose groups was a small increase in serum E2 levels seen.

In conclusion, pharmacokinetic results showed that absorption of FSH-CTP was much slower and the elimination half-life was twice as long as that of rFSH. Dose proportionality was shown in the dose range of 15–120 µg and the elimination half-life (ranging from 60 to 75 h) appeared to be dose independent. The preparation was safe and well tolerated. Pharmacodynamic effects of a single dose of 120 µg FSH-CTP were slightly less than those of 7 days administration of 150 or 225 IU rFSH. This long-acting FSH preparation offers the possibility of developing new treatment regimens with fewer injections, reducing patients’ discomfort in infertility therapy.

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References


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