A randomized three-way cross-over study in healthy pituitary-suppressed women to compare the bioavailability of human chorionic gonadotrophin (Pregnyl®) after intramuscular and subcutaneous administration

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The objective of this study was to compare the bioavailability of s.c. and i.m. administration of human chorionic gonadotrophin (HCG; Pregnyl®). In a randomized, single-centre, three-way cross-over study, 18 healthy pituitary-suppressed volunteers were assigned to single HCG injections of 5000 and 10 000 IU i.m. and 10 000 IU s.c. Rate (Cₘₐₓ, tₘₐₓ) and extent (area under curve from zero to infinity (AUCₚ₋ₚᵢₙ)) of absorption of HCG were determined. Serum immunoactive HCG increased from 0.4–0.5 IU/l at baseline to mean peak concentrations, which were reached 20 h after injection of 156 IU/l with 5000 IU i.m., of 307 IU/l with 10 000 IU i.m. and of 339 IU/l with 10 000 IU s.c. Eight days after administration, <10% of the maximum HCG activity was found for each regimen. The elimination half-life (tᵢₙ) was on average 32–33 h, irrespective of the treatment regimen. Intramuscular and s.c. injections of 10 000 IU HCG were bioequivalent with respect to AUCₚ₋ₚᵢₙ. The Cₘₐₓ and tₘₐₓ were also similar between the two administration routes but bioequivalence could not be proven due to intersubject variability. Intramuscular doses of 5000 IU and 10 000 IU HCG were dose-proportional. Since s.c. HCG is bioequivalent to i.m. HCG with respect to extent of absorption (its major pharmacokinetic variable) and is well tolerated, the s.c. administration route may be effectively and safely used in assisted reproduction. Moreover, since s.c. injection can be performed by the patients themselves, acceptability may be enhanced.

Key words: bioequivalence/human chorionic gonadotrophin/intramuscular/pharmacokinetics/subcutaneous

Introduction

Human chorionic gonadotrophin (HCG) is produced by the trophoblast and is excreted in maternal urine during pregnancy. It is a peptide hormone that stimulates gonadal steroidogenesis by a mechanism similar to that of luteinizing hormone (LH) (Ren and Braunstein, 1992). Pregnyl® is an HCG preparation which is obtained from the urine of pregnant women and is used for ovulation induction after ovarian stimulation with follicle stimulating hormone (FSH)-containing preparations both in anovulatory infertility and in-vitro fertilization (IVF) (5000–10 000 IU as a single dose). HCG is also used for luteal phase support, especially if gonadotrophin-releasing hormone (GnRH) agonists have been used for pituitary suppression (1000–3000 IU, 2–3 times during the first 9 days following ovulation). In men, HCG is used for the treatment of hypogonadal hypogonadism (1000–2000 IU, 2–3 times a week), cryptorchidism (250–1500 IU twice weekly for 6 weeks) and delayed puberty associated with insufficient gonadotrophic pituitary function (1500 IU, 2–3 times a week for at least 6 months).

Urinary gonadotrophins are usually given using the i.m. route of administration. As a rule, such injections are given by skillful spouses, qualified nurses, general practitioners or other physicians, often requiring frequent visits to the clinic. Compared to the i.m. route, the s.c. route has the main advantage that self-administration is feasible, thus limiting the number of visits to the clinic. Until recently, it was believed that urinary gonadotrophins could not be injected s.c., since the administration of these preparations with their relatively high amounts of impurities via this route may induce undesirable local adverse reactions (Le Cotonnec et al., 1993). Although allergic reactions have been described after s.c. injection of urinary gonadotrophins in animals (Biffoni et al., 1994), studies in humans suggest that the s.c. route is safe and that the incidence of local adverse reactions is low (Saal et al., 1991a, b; Jones and Darne, 1993; Jones et al., 1994; Out et al., 1996b, Schmutziguer et al., 1996). Thus, the bioequivalence issue of s.c. and i.m. administration of HCG (which has not previously been investigated in women) is important in clinical decision making when choosing an efficacious and safe administration route for these preparations. Moreover, as a result of an increasing shortage of HCG and the tendency to use lower HCG doses for assisted reproduction, it is useful to know how the pharmacokinetic behaviour of i.m. HCG administration of 5000 IU compares to 10 000 IU.

The objectives of the study were to assess the bioequivalence of an s.c. and i.m. administered HCG preparation as well as to assess dose-proportionality of two i.m. administered HCG doses.

Materials and methods

The study was designed as a single-centre, randomized, single-dose, three-way cross-over study in healthy female volunteers and was performed at the TNO Nutrition and Food Research Institute, Zeist (The Netherlands). The study was approved by the Medical Ethics Committee of the study centre and each subject had given her written informed consent before participating in the study. The study was
conducted in compliance with the Declaration of Helsinki as well as with local rules and regulations.

The study included 18 healthy female volunteers who received financial compensation for their participation in the study. This number of subjects is considered to have sufficient power, based on prior experiences in studies with a similar design (Out et al., 1996b; Huisman et al., 1997). Subjects were assigned to one of the three starter groups using randomization lists. In order to exclude possible interference by endogenous gonadotrophins, all women were pituitary-suppressed by the high-dose combined oral contraceptive (Lyndiol®; NV Organon, Oss, The Netherlands) during the whole study period (Huisman et al., 1997).

The main inclusion criteria were: healthy women between 18 and 35 years using combined oral contraceptives and of normal body weight. Main exclusion criteria were: pregnancy or lactation, a history of or current endocrine abnormalities, contraindications to the use of combined oral contraceptives or gonadotrophins, hypertension, concomitant use of medication within 4 weeks prior to the study, smoking >10 cigarettes per day, laboratory values indicative of physical illness and use of investigational drugs within 3 months prior to the study.

Before entering the study, the subjects’ eligibility was assessed by means of a physical examination and gynaecological examination and an HCG test to exclude pregnancy. A similar examination was performed at the end of the study period.

Lyndiol (50 µg ethinyl oestradiol and 2.5 mg lynestrenol per tablet) is a high-dose oral contraceptive preparation supplied in strips containing 22 tablets each, and was taken during the entire study period. Pregnyl® (NV Organon, Oss, The Netherlands) (HCG 5000 IU) was supplied as a freeze-dried, lyophilized powder in ampoules. For injection, each ampoule was reconstituted in a 9 g/l NaCl solution. All i.m. injections (HCG 5000 IU and 10 000 IU) were administered in the upper lateral quadrant of the gluteus maximus muscle, whereas all s.c. injections (HCG 10 000 IU) were given in the umbilical region.

Each subject started Lyndiol on the first or second day of the start of the first menstrual period following the pre-study screening. The first HCG injection was performed after at least one Lyndiol tablet had been taken, usually between 8.00 and 9.00 a.m. on the first days of each study period. Blood was sampled for HCG assessments ~15 min pre-dose and 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, 30, 36, 48, 72, 96, 120, 144, 168, and 192 h post-dose. After sampling, serum was prepared and stored frozen until HCG assay was performed. Serum HCG immunoactivity was analysed on the AIA-600 Enzyme Immuno Analyzer using AIA-PACK HCG kits (Tosoh Corp., CA, USA). The accuracy and within-run and between-run precision were within the limits required for validation and the lower limit of detection of the assay was 1 IU/l.

For bioequivalence testing, the maximum concentration (C max) and area under curve from zero to infinity (AUC 0–∞) values have been adjusted (‘normalized’) by dividing these figures by the HCG dose administered. Based on serum immunoactive HCG concentrations, the following pharmacokinetic parameters were calculated: C max, normalized C max (nC max), maximum time (t max), normalized AUC 0–∞ (nAUC 0–∞) and elimination half-life (t 1/2). The C max and t max were taken from the measured serum concentration data after the last injection. The t 1/2 was calculated using log-linear regression on the HCG values of the samples taken at 72, 96, 120, 144, 168 and 192 h after each dosing. The AUC 0–∞ after dosing was calculated as: AUC 0–∞ = AUC 0–t 1/2 + AUC t 1/2–∞, where t 1/2 is the last measurable data point. AUC 0–t 1/2 was calculated by means of the linear trapezoidal rule and AUC t 1/2–∞ as C tz /β, where C tz is the best-fitted concentration at time t 1/2 and β = ln 2/t 1/2.

The bioequivalence of the two administration routes was tested using guidelines issued by the International Harmonization and Consensus DIA Meeting on bioavailability testing and standards (Cartwright, 1991) and a detailed description of application of these guidelines to gonadotrophin preparations has recently been published (Huisman et al., 1997).

Results

Eighteen out of the 20 eligible female subjects entered the study and none of them discontinued prematurely. They had a mean age of 21.2 ± 2.0 years (range 18–26 years), a mean body weight of 65.1 ± 5.8 kg (range 54.1–73.6 kg) and a mean height of 172 ± 6 cm (range 157–179 cm).

In general, the i.m. and s.c. HCG injections were well tolerated. With each administration route, occasionally injection site pain was observed, starting several hours after administration and lasting up to several days. In total, 17 cases of injection site pain were reported over a total of 54 injections (31.5%) in nine out of 18 subjects. Other mentioned adverse experiences such as headache, breast tenderness, irregular bleeding and mood swings were most likely due to oral contraceptive intake. Serious adverse experiences were not reported.

Serum immunoactive HCG concentrations increased from 0.4–0.5 IU/l at baseline to mean peak concentrations reached 20 h after injection of 156 IU/l (range: 89–219 IU/l) with 5000 IU i.m., of 307 IU/l (range: 161–517 IU/l) with 10 000 IU i.m. and of 339 IU/l (range: 233–586 IU/l) with 10 000 IU s.c. Eight days after administration, <10% of the maximum HCG activity was found for each regimen (Figure 1). The t 1/2 was on average 32–33 h, irrespective of the treatment regimen. No treatment-order effects were observed.

The t 1/2 and normalized AUC 0–∞ showed bioequivalence for i.m. and s.c. HCG 10 000 IU. Due to a large intersubject variation, bioequivalence could not be proven on basis of nC max and t max (Table 1). For i.m. administration, dose proportionality was observed for HCG 5000 IU and HCG 10 000 IU as judged by the nAUC 0–∞ and nC max . No difference in
Dose proportionality testing of i.m. human chorionic gonadotrophin (HCG) 5000 IU and 10 000 IU (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HCG 5000 IU i.m.</th>
<th>HCG 10 000 IU i.m. (µ5000-µ10 000)</th>
<th>µs.c./µi.m.</th>
<th>90% CI</th>
<th>95% CI</th>
<th>Bioequivalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>(nC_{\text{max}}) (IU/l)</td>
<td>0.03122 ± 0.00886</td>
<td>0.03070 ± 0.00965</td>
<td>1.022</td>
<td>0.885–1.180</td>
<td>0.859–1.215</td>
<td>Yes</td>
</tr>
<tr>
<td>(nAUC_{0-\infty}) (IU.h/l)</td>
<td>2.745 ± 0.590</td>
<td>2.813 ± 0.587</td>
<td>0.977</td>
<td>0.894–1.067</td>
<td>0.878–1.086</td>
<td>Yes</td>
</tr>
<tr>
<td>(t_{\text{max}}) (h)</td>
<td>20.23 ± 11.23</td>
<td>20.78 ± 9.68</td>
<td>(-0.01667)</td>
<td>-4.992–3.000</td>
<td>-6.000–4.017</td>
<td>Not proven</td>
</tr>
<tr>
<td>(t_{\text{1/2}}) (h)</td>
<td>32.83 ± 3.37</td>
<td>33.55 ± 4.14</td>
<td>0.981</td>
<td>0.936–1.028</td>
<td>0.928–1.038</td>
<td>Yes</td>
</tr>
</tbody>
</table>

\(nC_{\text{max}}\) = normalized maximum concentration.
\(nAUC_{0-\infty}\) = normalized area under curve from zero to infinity.
\(t_{\text{max}}\) = maximum time.
\(t_{\text{1/2}}\) = elimination half-life.
\(\mu\) = geometric mean.

Bioequivalence of intramuscular and subcutaneous HCG

Table I. Bioequivalence testing of s.c. and i.m. human chorionic gonadotrophin (HCG) 10 000 IU (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HCG 10 000 IU s.c.</th>
<th>HCG 10 000 IU i.m.</th>
<th>(\mu)s.c./(\mu)i.m. ((\mu)s.c.-(\mu)i.m.)</th>
<th>90% CI</th>
<th>95% CI</th>
<th>Bioequivalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>(nC_{\text{max}}) (IU/l)</td>
<td>0.03390 ± 0.00870</td>
<td>0.03070 ± 0.00965</td>
<td>1.123</td>
<td>0.972–1.297</td>
<td>0.944–1.335</td>
<td>Not proven</td>
</tr>
<tr>
<td>(nAUC_{0-\infty}) (IU.h/l)</td>
<td>3.048 ± 0.532</td>
<td>2.813 ± 0.587</td>
<td>1.092</td>
<td>0.999–1.193</td>
<td>0.982–1.214</td>
<td>Yes</td>
</tr>
<tr>
<td>(t_{\text{max}}) (h)</td>
<td>20.03 ± 8.30</td>
<td>20.78 ± 9.68</td>
<td>(-0.01667)</td>
<td>-6.675–4.992</td>
<td>-6.992–6.000</td>
<td>Not proven</td>
</tr>
<tr>
<td>(t_{\text{1/2}}) (h)</td>
<td>32.28 ± 3.80</td>
<td>33.55 ± 4.14</td>
<td>0.936</td>
<td>0.919–1.009</td>
<td>0.911–1.019</td>
<td>Yes</td>
</tr>
</tbody>
</table>

\(nC_{\text{max}}\) = normalized maximum concentration.
\(nAUC_{0-\infty}\) = normalized area under curve from zero to infinity.
\(t_{\text{max}}\) = maximum time.
\(t_{\text{1/2}}\) = elimination half-life.
\(\mu\) = geometric mean.

Discussion

The current study was the first to compare the pharmacokinetics of HCG after s.c. and i.m. administration in women. The main finding was that i.m. and s.c. injections of 10 000 IU HCG were bioequivalent with respect to the extent of absorption (\(nAUC_{0-\infty}\)) and rate of absorption (\(nC_{\text{max}}\)), and no difference in \(t_{\text{1/2}}\) was found between these two doses. \(T_{\text{max}}\) was similar for each dose, but dose-proportionality could not be proven due to intersubject variability.

Urinary gonadotrophins have been used for decades in the treatment of female and male infertility and most of them are indicated for i.m. use. Although associated with relatively few adverse events (Verhoeoff et al., 1997), the i.m. administration route as well as the possible requirements for injections during the weekend and holidays may introduce practical problems such as frequent 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. Subcutaneous self-injection of gonadotrophins may therefore offer advantages for both patients (and their partners) and the hospital in terms of convenience and workload (Huisman et al., 1997).

The first published pharmacokinetic data on i.m. HCG were from a small study (two men and one woman) in which after i.m. administration of HCG 10 000 IU, a \(t_{\text{max}}\) of ~6 h was found, with a mean \(t_{\text{1/2}}\) of 30 h (Rizkallah et al., 1969). These findings were confirmed in two subsequent studies in men (Saez and Forest, 1979; Martikainen et al., 1980). In contrast, in a more recent study in women, a longer \(t_{\text{1/2}}\) of ~55 h was calculated after i.m. injection of 10 000 IU HCG (Damewood et al., 1989). The first study that compared the pharmacokinetics of s.c. and i.m. HCG was performed in healthy male volunteers (Saal et al., 1991a). In this study, after i.m. administration of 5000 IU HCG, a \(t_{\text{max}}\) of 6 h and a \(t_{\text{1/2}}\) of 31 h was reported, thereby confirming earlier findings (Rizkallah et al., 1969; Saez and Forest, 1979; Martikainen et al., 1980). However, in the study by Saal et al. (1991a) after s.c. administration of 5000 IU HCG, the \(t_{\text{max}}\) was prolonged to 16 h and the \(t_{\text{1/2}}\) to 38 h. Moreover, the \(C_{\text{max}}\) and AUC were significantly lower than after i.m. administration. In spite of these differences in HCG pharmacokinetics between i.m. and s.c. administration in males, similar testosterone responses were observed as measured by \(C_{\text{max}}\) and AUC, indicating that the clinical relevance of the observed differences in pharmacokinetics was only minor. Also, in another study in hypogonadal men, bioequivalence of s.c. and i.m. administration of HCG was investigated indirectly by measurement of the testosterone response (Jones et al., 1994). After administration of 5000 IU HCG by either route, concentrations of serum and salivary testosterone in response to HCG were comparable.
The investigators concluded that, from an efficacy point of view, there was no relevant difference between the administration routes. However, from an acceptability point of view the s.c. administration route might be preferred over the i.m. route, which was illustrated by subjects electing to continue treatment after the study on s.c. HCG as opposed to i.m. HCG.

The HCG doses used in the current study were based on the study by Saal et al. (1991a). In the present study, HCG was administered i.m. in doses of 5000 IU and 10 000 IU and s.c. in a dose of 10 000 IU. The s.c. dose was expected to yield values between those obtained from both i.m. administrations. However, in contrast to the study by Saal et al. (1991a), we did not find significant differences between i.m. and s.c. administration of 10 000 IU HCG.

When comparing our results after i.m. and s.c. administration of HCG (slight increases in nC_max and nAUC_0–inf as well as slight reductions of t_max and t_1/2) with studies in which the pharmacokinetics of other s.c. and i.m. administered gonadotrophins have been compared, it appears that conflicting results have been reported: in one report (Dobbs et al., 1994), the findings on s.c. and i.m. administered human menopausal gonadotrophin (HMG) in women were in line with the findings in males by Saal et al. (1991a), and thus in contrast to our findings. However, the pharmacokinetic behaviour of the gonadotrophin preparation may have been influenced by the fact that their study was performed in obese women. It was speculated that obese women as a result of a larger distribution volume may develop lower serum concentrations (Dobbs et al., 1994). In contrast, previous work in non-obese women on bioequivalence of s.c. and i.m. administration of two HMG preparations (Huisman et al., 1997) as well as of an FSH preparation (Le Cotonnec et al., 1993) are in good agreement with the findings from the current study.

Group sizes in this study were based on previous studies with a similar design (Out et al., 1996b; Huisman et al., 1997). It was demonstrated that these group sizes were adequate for calculating the bioequivalence of the two administration routes with respect to extent of absorption (nAUC_0–inf). However, the study also indicated that for proving bioequivalence of t_max, larger group sizes are probably needed.

Dose-proportionality of the pharmacokinetic parameters of single intramuscular doses of 5000 and 10 000 IU HCG was demonstrated for nC_max, nAUC_0–inf, and t_1/2, which is relevant in view of the tendency of prescribing lower HCG doses due to the increasing world-wide shortage of HCG. These results are consistent with linear pharmacokinetics over the dose range investigated, which is similar to the dose range that is normally used in assisted reproduction techniques. In addition, with a t_1/2 of ~32 h, it will take ~6 days before exogenous HCG is cleared from the circulation. This is especially relevant in women with an increased risk for ovarian hyperstimulation when the HCG dose is lowered or omitted.

In summary, since s.c. HCG is bioequivalent to i.m. HCG with respect to extent of absorption (its major pharmacokinetic variable) and is well tolerated, the s.c. administration route may effectively and safely be used in assisted reproduction. Moreover, since s.c. injection can be performed by the patients themselves, acceptability may be enhanced.

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