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ARTICLE

First-in-human trial assessing the pharmacokinetic-pharmacodynamic profile of a novel recombinant human chorionic gonadotropin in healthy women and men of reproductive age

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Abstract

The purpose of this first-in-human trial was to examine the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of a novel recombinant human chorionic gonadotropin (rhCG; FE 999302, choriogonadotropin beta) to support its clinical development for various therapeutic indications. The single and multiple dose PK of choriogonadotropin beta (CG beta) were evaluated in women and the single dose PK and PD of CG beta were compared to those of CG alfa in men. CG beta was safe and well-tolerated in all 84 healthy subjects. In women, the area under the curve (AUC) and the peak serum concentration (C_{max}) increased approximately dose proportionally following single and multiple doses of CG beta. The apparent clearance (CL/F) was ~ 0.5 L/h, the mean terminal half-life ($t_{½}$) ~ 45 h and the apparent distribution volume (V_z/F) ~ 30 L. After single administration in men, the mean AUC was 1.5-fold greater for CG beta than for CG alfa. Mean C_{max} and V_z/F were comparable for the 2 preparations. In accordance with the differences in AUC, the CL/F was lower for CG beta (CL/F 0.5 vs. 0.8 L/h), explained by a longer $t_{½}$ (47 vs. 32 h). Serum testosterone levels induced by a single dose rhCG reflected the PK profiles with a slight delay, resulting in 59% higher AUC for CG beta. The PK parameters for CG beta were comparable in men and in women. In conclusion, the PK differs between the two rhCG preparations, causing higher exposure and a higher PD response for CG beta, which may require relatively lower therapeutic doses.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Recombinant human chorionic gonadotropin (hCG) is indicated for the treatment of male or female infertility and administered by single or multiple subcutaneous injections.

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WHAT QUESTION DID THIS STUDY ADDRESS?

A new recombinant hCG (rhCG; choriogonadotropin [CG] beta) produced by a human-derived cell line (PER.C6) is currently in clinical development. The amino acid sequence of the α- and β-chains are identical to the natural sequences and also to that of rhCG expressed by Chinese Hamster Ovary (CHO) cell line (CG alfa), but the glycosylation provided by the PER.C6 and CHO cells is different. In this trial, the pharmacokinetics (PK) of choriogonadotropin beta were assessed in women and men and the PKs and pharmacodynamics (PDs) were compared in men to those of CG alfa.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

It is concluded that the PK of the two rhCG preparations are different, due to a slower clearance of CG beta resulting in a higher PD response.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Further development of CG beta may require lower doses of this potent hCG compared to current therapeutic hCG preparations.

INTRODUCTION

Human chorionic gonadotropin (hCG) is a glycoprotein hormone that is produced by the pituitary in small amounts in nonpregnant women, men, and menopausal women whereas large amounts are produced by the placenta of pregnant women.[1,2] During early pregnancy, hCG is first expressed by the blastocyst before implantation and is increasingly produced after implantation by the syncytiotrophoblast. During the first trimester of pregnancy, hCG is produced in increasing amounts up to 10th week of gestation and then decreases gradually.[3] Because intact hCG is cleared by the kidneys, hCG may be isolated from the urine of women and used for the manufacturing of therapeutic preparations.[4,5]

The hCG consists of a 92 amino acid single α-subunit, which is common to all the pituitary glycoprotein hormones, and a specific β-subunit of 145 amino acids. Each subunit is post-translationally modified by the addition of complex carbohydrate moieties. The alpha subunit contains 2-N-linked glycosylation sites at amino acids 52 and 78 and the beta subunit contains 2-N-linked glycosylation sites at amino acids 13 and 30 and 4 O-linked glycosylation sites at amino acids 121, 127, 132, and 138.[6,7] The hCG and luteinizing hormone (LH) shows similar molecular structures and interact with the same LH/chorionic gonadotropin (CG) receptor.[8] As a result of this similarity to LH, hCG is used pharmacologically in a number of clinical indications. In women, hCG is used to induce final follicular maturation following controlled ovarian stimulation or to induce ovulation in anovulatory women.[9] In men with hypogonadotropic hypogonadism, hCG is given to induce and maintain spermatogenesis.

To date, there is only one approved recombinant hCG (rhCG) preparation (CG alfa) which is expressed by a Chinese Hamster Ovary (CHO) cell-line and the pharmacokinetics (PK) are similar to those of urinary hCG.[10,11]

CG beta (FE 999302) is a novel rhCG that has been produced by a human cell line (PER.C6). The amino acid sequence of the α- and β-chains of CG beta are identical to those of endogenous hCG and CG alfa. Glycosylation of both natural and recombinant hCG is highly complex and may contain a wide range of structures.[12] The glycosylation of rhCG reflects the range of glycosyl-transferases present in the host cell line and is known to differ between rhCG products produced by different cell lines.[13]

PER.C6 and CHO cell lines are both used for production of recombinant follicle-stimulating hormone (rFSH), with follitropin alfa expressed by a CHO cell line and follitropin delta expressed by the PER.C6 cell line. Investigations show that the preparations of rFSH from the PER.C6 human cell line and a CHO cell line display important differences in PK and pharmacodynamic (PD) properties.[14] These differences include consistently higher exposure, longer time to peak serum concentration (C_{max}), and longer terminal half-life ($t_{½}$) of follitropin delta after a single administration, and longer $t_{½}$ at steady-state after repeated administrations, compared with follitropin alfa. A significantly lower clearance of follitropin delta compared with that of follitropin alfa was also well measured. Based on these differences, which can be attributed to the glycosylation profile, it may be anticipated that the PK and PD properties of rhCG expressed by a human cell line and by a CHO cell line will also be dissimilar.

To examine the safety, PK, and PD of CG beta, the first-in-human trial comprised three parts conducted sequentially and included healthy women using oral contraceptives and healthy men downregulated with GnRH agonist. The single and multiple dose PK of CG beta were first evaluated in healthy women and then the single dose PK and PD were compared to those of

CG alfa in healthy men. The goal of this research was to establish the PK and PD of CG beta in women and men over a broad dose-range in order to allow further development of CG beta for any potential therapeutic indication.

METHODS

Participants

This first-in-human trial of CG beta included 84 women and men. Eligible participants were women 18–40 years of age or men 18–50 years of age with a body mass index (BMI) of 18–29 kg/m^2. All participants were healthy according to medical history, physical examination (including gynecological examination in women), a 12-lead electrocardiogram (ECG), and clinical laboratory profiles of blood and urine. Written informed consent was obtained from all subjects prior to inclusion in the trial, which was conducted in accordance with the Declaration of Helsinki and International Council for Harmonization–Good Clinical Practice. The trial was approved by the Ethical Committee of the Bavarian Chamber of Physicians, Germany.

Study design

This trial was composed of 3 parts, including only women in parts 1 and 2 and men in part 3. All women were required to have used combined oral contraceptive (ethinylestradiol content ≥0.015 mg) or combined contraceptive vaginal ring for at least three cycles prior to trial inclusion. All women were switched to Yasmin (Bayer) contraceptive tablets 14 days prior to CG beta administration and this contraceptive was taken daily throughout the study period. Men were downregulated with a depot GnRH agonist (triptorelin, Decapeptyl, Ferring Pharmaceuticals) to suppress endogenous hormone production.

Part 1

The first part of the trial had a double-blind, placebo-controlled and randomized single ascending dose design and included 35 women. Divided in 5 cohorts (5 active treatment, and 2 placebo in each cohort), 25 women were dosed with a single dose of CG beta and 10 women were dosed with placebo. The dose levels of CG beta were 4, 16, 64, 128, and 256 µg. All doses were administered as single subcutaneous injections in the abdomen. Blood samples for measurement of serum hCG concentrations were obtained immediately before administration of CG beta or placebo and at 2, 5, 8, 10, 12, 14, 16, 24, 36, 48, 72, 96, 120, 144, 168, 216, and 264 h after administration.

Part 2

The second part of the trial had a double-blind, placebo-controlled, and randomized multiple ascending dose design and included 16 women. Divided into 2 cohorts (6 active treatment, and 2 placebo in each cohort), 12 women were dosed daily with CG beta and 4 women were dosed with placebo. The daily CG beta dose levels were 8 and 16 µg administered as single subcutaneous injections in the abdomen for 10 consecutive days. Blood samples for measurement of serum hCG concentrations were obtained immediately before administration of each CG beta or placebo doses, and then at 2, 5, 8, 10, 12, 14, 16, 24, 36, 48, 72, 96, 120, 144, 168, 216, and 264 h after the last dose.

Part 3

The last part of the trial had an open randomized 2-way crossover design comparing the PK and the testosterone release after administration of CG beta and CG alfa (Ovitrelle; Merck Serono) in 33 downregulated men. All men received 3 doses of 3.75 mg Decapeptyl in order to downregulate the pituitary-gonadal axis and suppress testosterone to less than or equal to 1 ng/ml and LH to less than or equal to 2.5 IU/ml at the time of rhCG administration. Two doses of Decapeptyl were administered prior to the first drug administration on days −28 and −10 and a third dose was given on day 12 after the first dose of rhCG. A single dose of 125 µg rhCG of each preparation was administered s.c. in a crossover design with the 2 treatment periods ~ 3 weeks apart. Blood samples for measurement of serum hCG concentrations were obtained immediately before drug administration, and then at 4, 8, 12, 14, 16, 24, 36, 48, 72, 96, 120, 168, 216, and 264 h after each administration. Blood samples for measurement of serum testosterone concentrations were collected immediately before drug administration and then 4, 8, 12, 16, 24, 48, 72, 96, 120, 168, 216, and 264 h after each administration.

Safety and tolerability

Safety and tolerability were assessed by monitoring of adverse events (AEs), injection site reactions, clinical laboratory assessments [clinical chemistry, hematology, and urinalysis], physical examination, vital signs [blood pressure, pulse, and body temperature], 12-lead ECG and—in women—transvaginal ultrasonography. The summarized AEs are those reported during the treatment phase (i.e., from administration of rhCG until the last assessment 11 days after the last dose).

Bioanalytical methods

Serum rhCG levels were measured using a sandwich immunoassay, comprising a monoclonal mouse anti-hCG beta 2 as capture antibody and a ruthenium-labeled monoclonal mouse anti-hCG Holo C3 as detection antibody with electrochemiluminescence (ECL) detection (Meso Scale Discovery system). The analytical standard used was CG beta for quantification of CG beta, and CG alfa for quantification of CG alfa, and the analytical range was 0.100–12.0 ng/ml serum (up to 240 ng/ml with extended dilution). Interassay precision was less than or equal to 9%, and intra-assay precision was less than or equal to 6%, in the main method validation, as calculated using analysis of variance (ANOVA). In order to ensure equivalent exposure CG alfa was quantitated by amino acid analysis independent of the label values.

Analysis of serum concentrations of testosterone was performed by means of a validated liquid chromatography tandem mass spectrometry method.

The analyses for antibodies against rhCG were performed using a validated bioanalytical method. The method validations were designed to follow the principles stated in Shankar et al. and the regulatory guideline.[15,16] The method was a semihomogenous bridging assay using ECL as the detection method. The trial samples were analyzed using a tiered approach. All samples (study samples and controls) were analyzed as duplicates and the mean signal was used for determination of results.

Statistical analyses

The PK, PD, and safety were summarized using descriptive statistics.

Women receiving a single dose of 4 μg CG beta had serum hCG concentrations below the limit of quantification in 4 of the 5 women. Therefore, it was not possible to calculate any meaningful PK variables for this dose group. In contrast, all serum hCG concentrations from other women and all men were included in the PK calculations.

The PK and PD parameters were calculated by noncompartmental analysis using the software Phoenix WinNonlin (Pharsight Corporation). The PD parameters were calculated for baseline corrected data, assuming a constant background testosterone concentration after downregulation. The relation between body weight (BW) and exposure was investigating for area under the curve (AUC) and C_{max} by fitting the function k/BW^c to data using linear regression after log-transformation. Dose adjusted exposure data from all subjects in all three parts were used for this investigation. If the exponent c is different from 0, then the exposure is related to BW. If c = 1, the exposure is inversely proportional to BW. Analysis of dose proportionality for AUC and C_{max} was based on the single dose groups 16 to 256 μg CG beta. The slope (beta) was estimated from the model log(parameter) = ln(alpha) + beta * ln(dose). A slope of 1 corresponds to dose proportionality.

Comparison of PK and PD parameters between CG beta and CG alfa in part three of the trial were performed using ANOVA on log-transformed parameters, including factors for drug, period, and subject. Estimated ratios and 90% confidence intervals (CIs) were derived from the model and back-transformation of log-transformed differences. The statistical analyses were performed using the software SAS.

RESULTS

Thirty-five healthy women aged between 18 and 40 years were randomized and dosed for the single dose PK investigation (part 1 of the trial), 7 in each treatment group. The mean BW was 65.9 kg with a range from 50.7 to 90.6 kg, and mean BMI was 23.9 kg/m^2 with a range from 19.1 to 28.9 kg/m^2. Overall, the treatment groups were similar with respect to demographic parameters.

For the repeated dose PK investigation (part 2 of the trial), 16 women between 19 and 40 years of age were randomized and dosed, 8 in each treatment group. The mean BW was 64.7 kg with a range from 55.0 to 81.4 kg, and mean BMI was 23.4 kg/m^2 with a range from 19.7 to 27.2 kg/m^2. Overall, the treatment groups were similar with respect to demographic and baseline characteristics.

Thirty-three healthy men between 18 and 50 years of age were included in the single dose PK and PD investigations (part 3 of the trial). The mean body weight was 82.6 kg with a range from 59.3 to 96.0 kg, and mean BMI was 25.3 kg/m^2 with a range from 19.9 to 29.0 kg/m^2.

Part 1: Single dose PK in women

The mean serum concentrations of CG beta after single dosing are shown in Figure 1.

After administration of CG beta, serum concentrations increased until reaching the maximal concentration at 24 h (median) with a range of 2 to 48 h. The geometric mean C_{max} ranged from 0.3 to 7.7 ng/ml after single doses of 16, 64, 128, and 256 μg CG beta. Subsequently, the concentrations declined with a geometric mean $t_{½}$ across the 4 evaluable doses of 45 h (percent coefficient of variation [CV%]: 18%). The concentrations were approximately back to baseline level 11 days after the administration of CG beta. The geometric mean values of apparent total clearance and apparent volume of distribution were estimated to 0.48 L/h (CV%: 30%) and 31 L (CV%: 31%), respectively, across the evaluable doses.

The AUC and C_{max} were approximately dose proportional within the analyzed dose range 16–256 μg. The slope (beta)

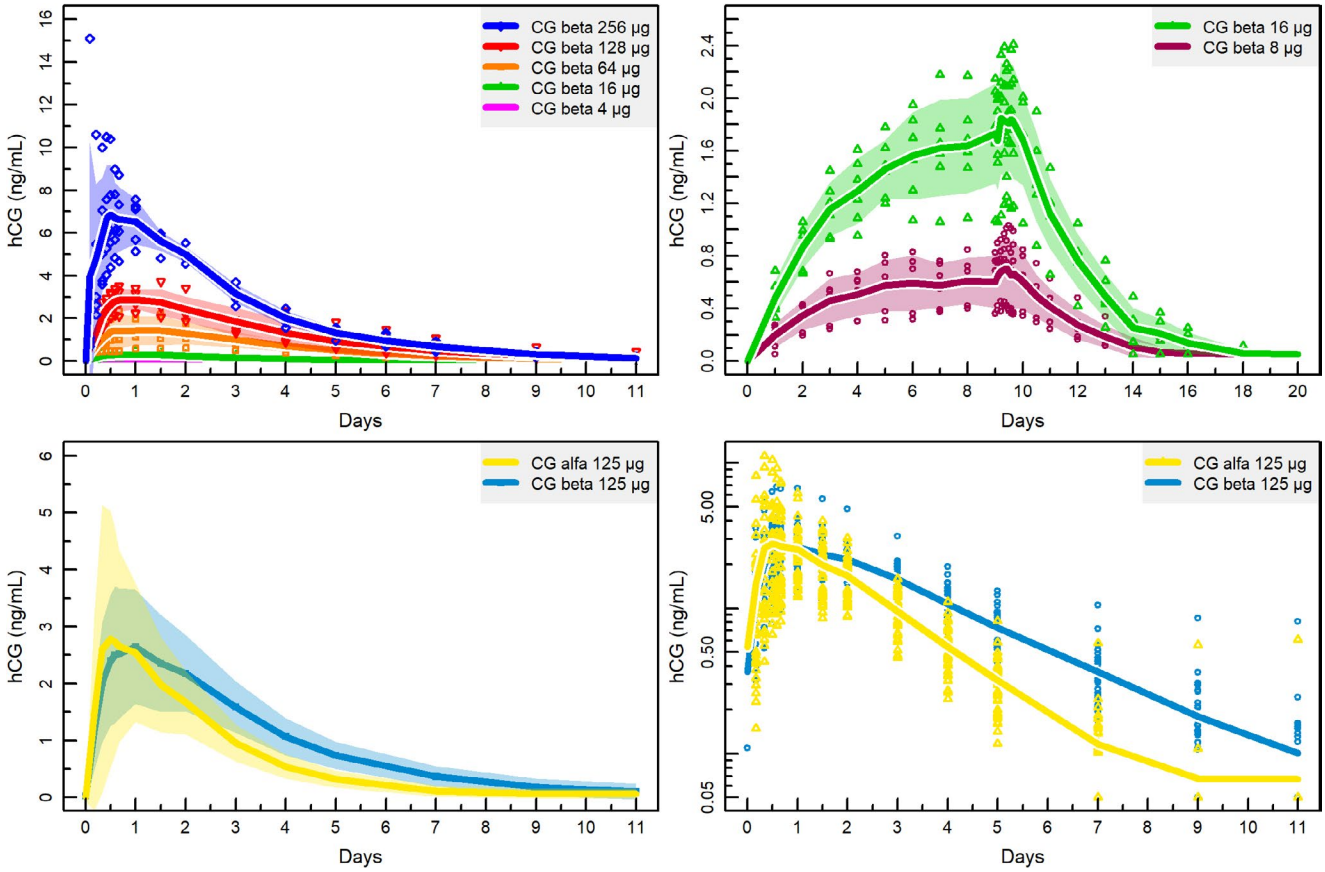

FIGURE 1 Time course of mean serum concentrations after single and multiple s.c. administrations of CG beta and CG alfa to women and men in parts 1 to 3 of the trial. Individual serum concentrations are shown with dots and the arithmetic mean with solid lines. Standard deviation is shown with shaded areas. The upper plot to the left shows the serum concentrations after single administration of CG beta to women in part 1 of the trial. The upper plot to the right shows the serum concentrations after multiple administration of CG beta to women for 10 days in part 2 of the trial. The lower plot to the left shows the serum concentrations after single administration of CG beta and CG alfa to men in part 3 of the trial. The lower plot to the right shows the serum concentrations on a logarithmic scale after single administration of CG beta and CG alfa to men in part 3 of the trial. CG, chorionic gonadotropin; hCG, human chorionic gonadotropin

was estimated to 1.14 (95% CI = 1.00–1.28) for AUC and 1.20 (95% CI = 1.01–1.38) for C_{max}.

Part 2: Multiple dose PK in women

The mean serum concentrations of CG beta after single dosing are shown in Figure 1.

Following the daily administration of CG beta over 10 days, the trough concentration increased and reached steady-state after 6–7 days in the 8 µg group and after 7–8 days in the 16 µg group. The median time for reaching maximal serum CG beta concentrations after the last CG beta dose was 10 h (range 5–16 h) after multiple dosing. The geometric mean C_{max} values were 0.69 ng/ml (CV%: 32%) in the 8 µg dose group and 1.9 ng/ml (CV%: 21%) in the 16 µg dose group. Two subjects in the 8 µg group showed substantially lower exposure compared to the rest of the subjects in this dose cohort. The geometric mean $t_{½}$ across the 2 doses was 42 h (CV%: 15). The geometric mean values for the apparent total clearance and apparent volume of distribution of CG beta were 0.45 L/h (CV%: 40%) and 27 L (CV%: 50%), respectively.

Part 3 Single dose PK in men

The mean serum concentrations of CG beta and CG alfa after single dosing are shown in Figure 1.

The average time taken for the mean hCG concentration to reach C_{max} after a single injection of 125 µg CG beta compared to a single injection of 125 µg CG alfa was around 24 h for both compounds. The geometric mean serum C_{max} were also comparable being 2.59 ng/ml (CV%: 40%) after CG beta administration and 2.59 ng/ml (CV%: 73%) after CG alfa administration. However, in spite of similar C_{max} values, exposure as determined by AUC_t was substantially different with the geometric mean AUC_t for CG beta being 50% (90%

CI = 1.36–1.65) greater compared to that for CG alfa. This difference was also reflected in the geometric mean half-life, which was 47 h after a single injection of 125 µg CG beta and 32 h after a single injection of 125 µg CG alfa and the geometric mean apparent total clearance, which was 0.50 L/h (CV%: 31%) after CG beta administration and 0.75 L/h (CV%: 42%) after CG alfa administration. The geometric mean apparent distribution volumes (V_z/F) were 34 L (CV%: 37%) and 35 L (CV%: 46%), respectively, after CG beta and CG alfa administration.

Comparison of PK results after a single dose to women and men

Mean serum CG beta concentrations after single s.c. injection of 128 µg CG beta to women and 125 µg CG beta to men are shown in Figure 2.

After single dose administration of 128 µg CG beta to 5 women and 125 µg CG beta to 33 men the PK profiles and PK parameters for CG beta were comparable.

Relationship between body weight and CG beta exposure

The association between BW and exposure in women and men is shown in Figure 2. Regardless of gender, both AUC and C_{max} decreased with increasing body weight. The power exponent for BW was 0.85 (95% CI = 0.36–1.35, $p = 0.0009$) for AUC and 1.12 (95% CI = 0.61–1.63, $p < 0.0001$) for C_{max}, indicating that both AUC and C_{max} declined approximately proportionally to the inverse of the BW.

Part 3: Single dose PD in men

The mean baseline corrected serum testosterone concentrations after single dosing of CG beta and CG alfa are shown in Figure 3.

The median time for reaching baseline corrected maximal testosterone concentration was 96 h (range 48–168 h) after a single s.c. injection of 125 µg CG beta and 72 h (range 48–120 h) after a single s.c. injection of 125 µg CG alfa with

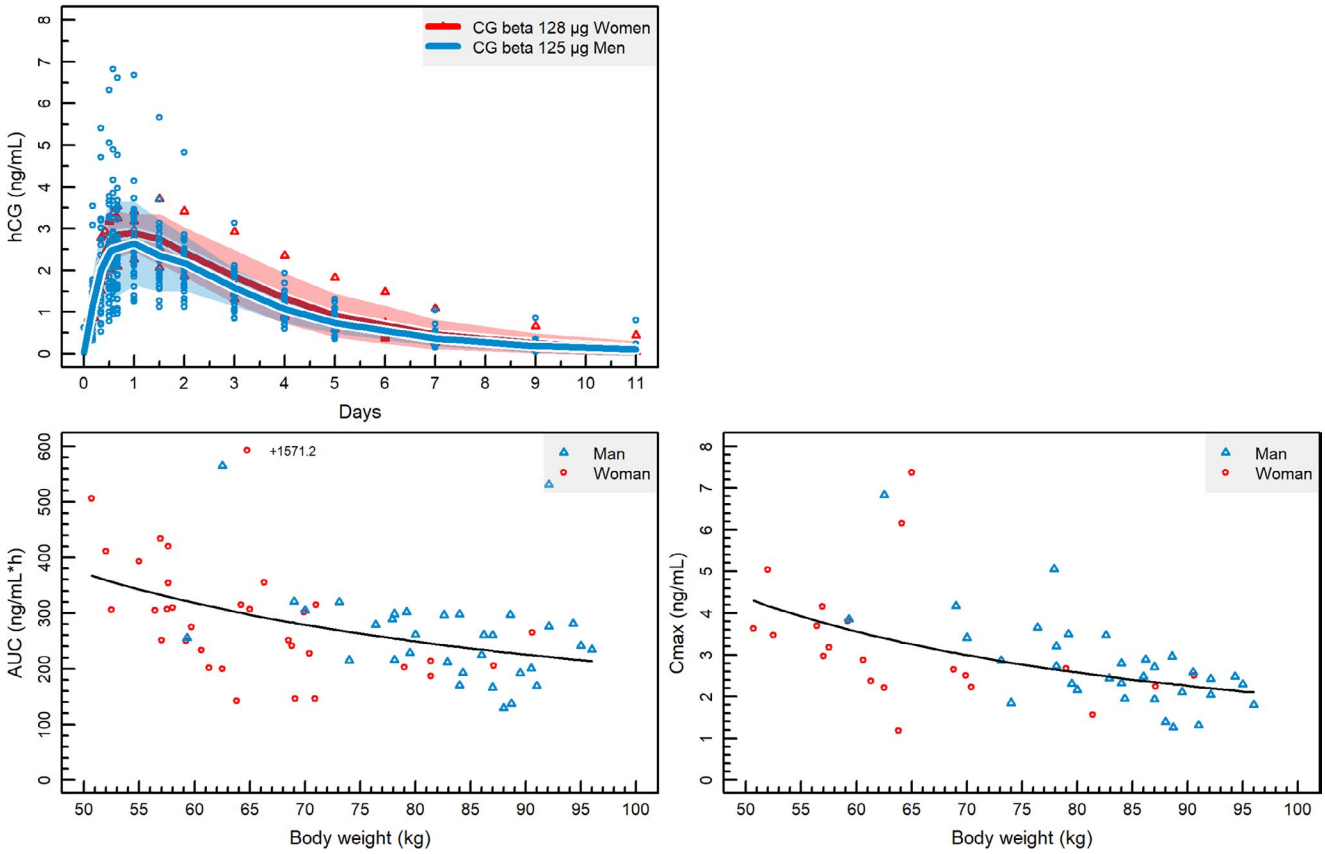

FIGURE 2 Exposure of CG beta in women and men. Upper plot: Time course of mean serum concentrations after single s.c. administration of 128 µg CG beta to women and 125 µg CG beta to men in part 1 and part 3 of the trial. Individual serum concentrations are shown with dots and the arithmetic mean with solid lines. Standard deviation is shown with shaded areas. Lower plots: Body weight influence on exposure by means of AUC and C_{max}. AUC and C_{max} values are dose normalized to 125 µg CG beta. Solid line represents fitted regression curve. AUC = $10335/BW^{0.85}$ ($p = 0.0009$) and $C_{max} = 349/BW^{1.12}$ ($p < .0001$). AUC, area under the curve; BW, body weight; CG, chorionic gonadotropin; C_{max}, peak plasma concentration; hCG, human chorionic gonadotropin

geometric mean testosterone plasma C_{max} concentrations of 7.1 ng/ml (CV%: 30%) and 6.7 ng/ml (CV%: 32%), respectively. In accordance with the concentration profiles and exposure of CG beta and CG alfa, the testosterone AUC_t was 1.6–fold (90% CI = 1.50–1.68) greater after administration with CG beta than with CG alfa.

A summary of the estimated PK parameters of CG beta in women and the estimated PK and PD parameters of CG beta and CG alfa in men is shown in Table 1 and Table 2, respectively.

Safety

CG beta was well-tolerated in both women and men after single or multiple s.c. injections. No severe or serious AEs occurred, no AE led to discontinuation of the trial, and none of the subjects developed antibodies against CG beta.

In Part 1, 21 AEs were reported by 12 women (48%) on active treatment and 7 AEs were reported by 5 women (50%) on placebo. There were no apparent dose-related trends in AE frequency. In part 2, there were 12 AEs in 6 women (100%) in the 8 µg group, 35 AEs in 5 women (83%) in the 16 µg group, and 12 AEs in 4 women (100%) in the placebo group. The most frequently reported AEs in women on active treatment were nausea, headache, and uterine spotting. In part 3, the frequency of AEs in downregulated men was comparable in the 2 treatments (i.e., 22 AEs occurred in 15 men [45%] after CG beta treatment, and 28 AEs occurred in 18 men (55%) after CG alfa treatment, without any apparent difference between the 2 groups. The most frequently reported AEs in men were hot flush and headache. An overview of AEs reasonably possibly related to treatment is provided in the Supplementary Tables S1–S3.

In part 3, two downregulated men experienced transient increases in alanine aminotransferase and aspartate aminotransferase in the second treatment period; in one subject, the liver enzyme increases occurred after administration of CG alfa, in the other subject they occurred prior to and after administration of CG beta. There were no other clinically significant findings or apparent dose-related trends in physical examination, vital signs, ECG, transvaginal ultrasounds, or safety laboratory data after either single or repeated administrations in women and men.

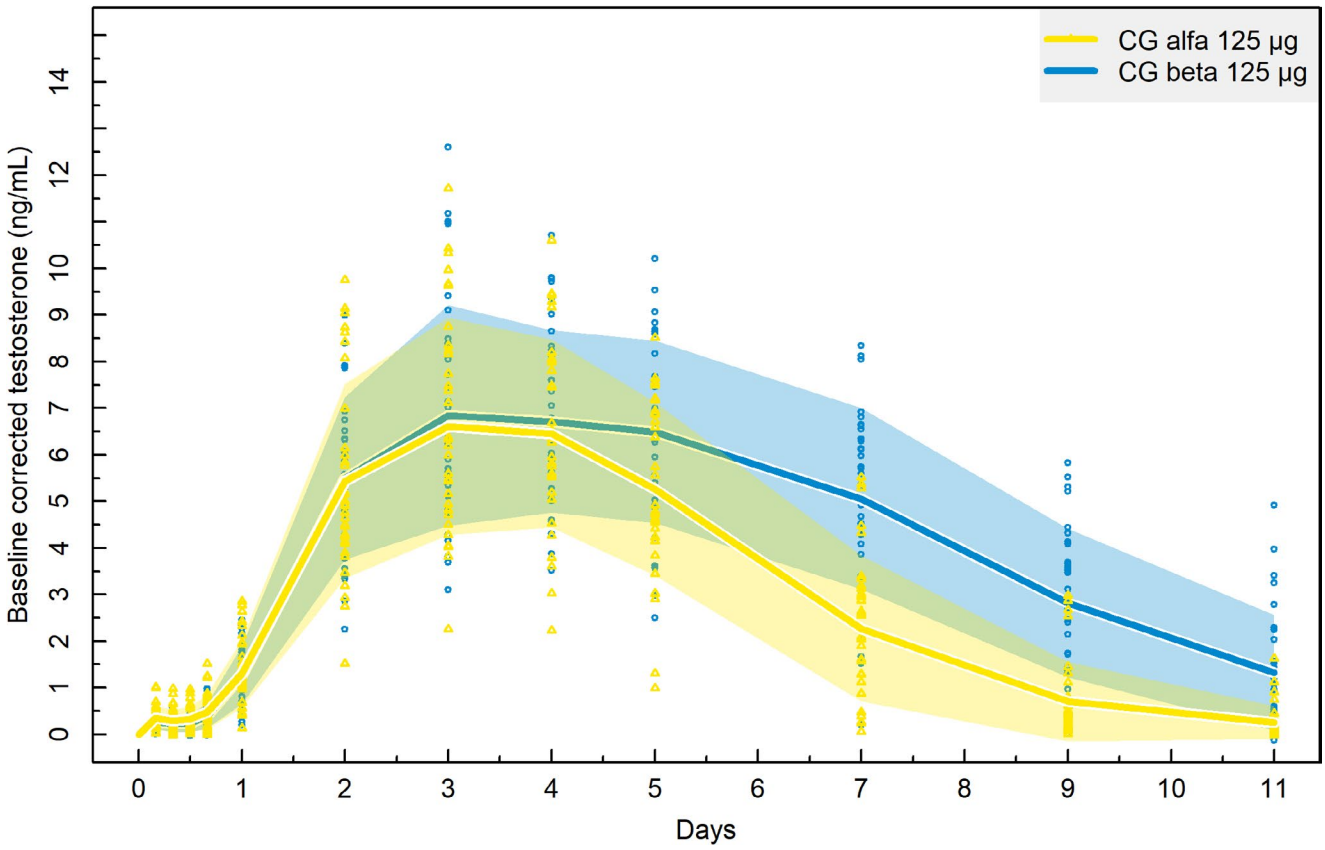

FIGURE 3 Time course of baseline corrected serum testosterone concentrations after single s.c. administration of CG beta and CG alfa to men in part 3 of the trial. Individual serum concentrations are shown with dots and the arithmetic mean with solid lines. Standard deviation is shown with shaded areas. CG, chorionic gonadotropin

TABLE 1 Summary of PK parameters for CG beta after single and multiple administration to women in part 1 and part 2 of the trial

PK parameters: Single dose of CG beta administration to women	**CG beta 16 µg** ***N*** **= 5**	**CG beta 64 µg** ***N*** **= 5**	**CG beta 128 µg** ***N*** **= 5**	**CG beta 256 µg** ***N*** **= 5**
AUC_{inf} (h*ng/ml)[a]	27.8 (22)	144 (40)	288 (41)	563 (10)
AUC_t (h*ng/ml)[a]	18.4 (32)	132 (42)	275 (38)	553 (10)
C_{max} (ng/ml)[a]	0.305 (28)	1.37 (51)	2.94 (18)	7.74 (47)
T_{max} (h)[b]	24 [14; 36]	24 [14; 48]	24 [16; 36]	16 [2; 24]
$t_{1/2}$ (h)[a]	46.0 (16)	47.0 (11)	46.8 (28)	42.0 (13)
CL/F (L/h)[a]	0.576 (17)	0.445 (50)	0.445 (31)	0.455 (10)
V_z/F (L)[a]	38.3 (27)	30.2 (48)	30.0 (18)	27.6 (12)
PK parameters: Multiple doses of CG beta administration for 10 days to women		**CG beta 8 µg** ***N*** **= 6**		**CG beta 16 µg** ***N*** **= 6**
AUC_τ (h*ng/ml)[a]		15.0 (35)		41.9 (22)
C_{max} (ng/ml)[a]		0.694 (32)		1.90 (21)
T_{max} (h)[b]		8 [5; 12]		11 [10; 16]
$t_{1/2}$ (h)[a]		43.5 (16)		40.8 (14)
CL/F (L/h)[a]		0.534 (40)		0.382 (26)
V_z/F (L)[a]		33.5 (51)		22.5 (21)

Abbreviations: AUC_{inf}, area under the concentration-time curve from time zero to infinity; AUC_t, area under the concentration-time curve from time zero to time of last measurable concentration (above the lower limit of quantification); AUC, area under the concentration-time curve during a dosing interval at steady-state; CG, chorionic gonadotropin; CL/F, apparent clearance; C_{max}, peak plasma concentration; PK, pharmacokinetic; $t_{1/2}$, terminal half-life; T_{max}, time to peak plasma concentration; V_z/F, apparent distribution volume.

[a]Geometric mean (coefficient of variation%),

[b]Median [range], *N* = number of subjects.

TABLE 2 Summary of PK and PD parameters for CG beta and CG alfa after single administration to men in part 3 of the trial

PK parameters: Single dose of CG beta and CG alfa administration to men	**CG beta, 125 µg** ***N*** **= 33**	**CG alfa, 125 µg** ***N*** **= 33**	**Ratio CG beta / CG alfa**		
			Estimate	**90% CI**	***p*** **value**
AUC_{inf} (h*ng/ml)[a]	249 (35)	166 (45)	1.50	1.36; 1.65	<0.00001
AUC_t (h*ng/ml)[a]	235 (32)	156 (46)	1.51	1.36; 1.67	<0.00001
C_{max} (ng/ml)[a]	2.59 (40)	2.59 (73)	1.00	0.87; 1.14	0.9961
T_{max} (h)[b]	24 [12; 48]	24 [8; 48]			
$t_{1/2}$ (h)[a]	47.1 (36)	32.3 (44)	1.46	1.41; 1.51	<0.00001
CL/F (L/h)[a]	0.503 (31)	0.754 (42)	0.67	0.61; 0.73	<0.00001
V_z/F (L)[a]	34.1 (37)	35.2 (46)	0.97	0.87; 1.08	0.6501
PD parameters: Single dose of CG beta and CG alfa administration to men	**CG beta, 125 µg** ***N*** **= 33** **Testosterone baseline corrected**	**CG alfa, 125 µg** ***N*** **= 33** **Testosterone baseline corrected**			
AUC_{inf} (h*ng/ml)[a]	1219 (40)	766 (35)	1.59	1.50;1.68	<0.00001
C_{max} (ng/ml)[a]	7.09 (30)	6.74 (32)	1.05	0.99; 1.11	0.1401
T_{max} (h)[b]	95.9 [48; 168]	72 [48; 120]			

Abbreviations: AUC_{inf}, area under the concentration-time curve from time zero to infinity; AUC_t, area under the concentration-time curve from time zero to time of last measurable concentration (above the lower limit of quantification); CG, chorionic gonadotropin; CI, confidence interval; CL/F, apparent clearance; C_{max}, peak plasma concentration; PD, pharmacodynamic; PK, pharmacokinetic; $t_{1/2}$, terminal half-life; T_{max}, time to peak plasma concentration; V_z/F, apparent distribution volume.

[a]Geometric mean (coefficient of variation%),

[b]Median [range], *N* = number of subjects.

DISCUSSION

The three-part design of this first-in-human trial of CG beta provides information on the safety of CG beta in healthy subjects, single and multiple dose PK in women, comparative single dose PK in men, and allows a comparison of the PK of CG beta between genders.

Single ascending doses up to 256 μg were safe and well-tolerated in women and the increases in serum CG beta levels were approximately proportional with dose. CG beta serum hCG concentrations were too low to calculate meaningful PK parameters after a single dose of 4 μg, but in the other dose groups from 16 to 256 μg the AUC and C_{max} of CG beta increased in an approximately dose proportional manner. The PK parameters $t_{½}$, apparent clearance (CL/F), and V_z/F were all similar across the dose range. The half-life of CG beta was longer (45 vs. 29 h), the CL/F (0.5 vs. 0.7 L/h) was lower and the V_z/F (31 vs. 29 L) was comparable, when compared to available literature data of CG alfa.[10,11] The difference in elimination rate between CG beta and CG alfa may be explained by the higher degree of sialylation of CG beta molecule including mono-, di-, tri-, as well as tetra-sialylation structures.[17]

Following multiple daily dosing of CG beta in women, serum hCG levels accumulated and reached approximate steady-state levels after 6–8 days. The estimates of $t_{½}$, CL/F, and V_z/F for CG beta after daily repeated administration were similar to the estimates obtained after a single dose of CG beta (42 vs. 45 h, 0.45 vs. 0.48 L/h, and 27 vs. 31 L, respectively). The median time of maximum plasma concentration (T_{max}) was naturally shorter after multiple administration (8–11 h) compared to single dose administration (16–24 h) as concentrations remaining from previous doses were declining exponentially. Thus, the shift in T_{max} was mainly caused by the slow elimination of CG beta in combination with the relatively short dosing interval of 24 h.

In part 3 of the trial, the rhCG dose administered to down-regulated men was 125 μg for both preparations. This choice of dose was based on previous experience with urinary hCG and published data for CG alfa.[10,18] A dose of 125 μg rhCG is high enough to give reliable comparative PK data and also induces sufficient testosterone production for comparative analysis (125 μg of CG alfa is approximately equivalent to 2500 IU as determined in the rat bioassay).[18] Administration of 125 μg CG beta and CG alfa to men resulted in considerably higher exposure (1.5-fold) to CG beta compared with CG alfa. In line with this, the estimated apparent clearance was lower and the half-life longer for CG beta when compared to CG alfa but the apparent volume of distribution after administration was similar between compounds. Despite the difference in exposure, the C_{max} for serum hCG concentration increased to similar levels indicating that the absorption rate is very similar, whereas the elimination is slower for CG beta as supported by the lower CL/F and in accord with the higher exposure. The PK data of CG alfa in part 3 are in good agreement with those previously published for CG alfa.[8,14] After single dose administration of 125 μg rhCG to men, the production of testosterone was higher (1.6-fold) following CG beta injection than after CG alfa injection. Maximum serum testosterone production was reached at 3 days after injection for both compounds but thereafter serum testosterone declined at a slower rate in the CG beta group than in the CG alfa group. Because the half-life of endogenous testosterone is relatively short, the slower testosterone decline reflects the longer half-life of CG beta.[19–21] Thus, the higher exposure and lower apparent clearance of CG beta when compared to CG alfa, resulted in sustained higher testosterone levels after CG beta administration.

The association between BW and exposure in both women and men indicated that regardless of gender, both AUC and C_{max} decreased with increasing body weight. Other studies have shown similar associations for urinary and other recombinant hCG preparations.[22–24]

Comparing the PK properties of CG beta after a single s.c. administration of 125 μg in men and 128 μg in women revealed very similar PK profiles without any apparent gender-specific characteristics. The slightly higher exposure in women is ascribed to their lower BW rather than to the marginally higher dose. The PK parameters were similar regardless of gender. The differences between rhCG expressed in a human cell line and in a CHO cell line were assessed in men only. However, because the PK differences of gonadotropins between men and women are known to be limited it is to be expected that the differences between CG beta and CG alfa observed in men can also be expected in women.[25]

The safety profile of CG beta in this trial was reassuring with rather few AEs, all of which were of mild or moderate intensity. The most frequent reported AE in women was headache, and in men was hot flush, the latter most likely related to their testosterone deficient status. Overall, the drug was well-tolerated and its potential immunogenicity seems low and in line with that reported for rFSH produced by the same cell line.[26]

In conclusion, CG beta has shown to be safe and well-tolerated both in women and men. The PK-PD profile of CG beta is different from CG alfa, and because the amino acid sequences are identical, it can be inferred that the glycosylation differences are responsible for the lower clearance of CG beta in comparison to CG alfa. Due to PK and PD differences, the potential therapeutic dose of CG beta is likely to be lower, in both women and men, than that for other hCG preparations.

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CONFLICT OF INTEREST

All authors are present employees of Ferring Pharmaceutics A/S and there are no other relationship or activities that could appear to have influenced the submitted work.

AUTHOR CONTRIBUTIONS

L.-E.B.K., C.H., K.B., and B.M. wrote the manuscript. L.-E.B.K., C.H., P.L., and B.M. designed the research. P.L. and L.-E.B.K. performed the research. L.-E.B.K., P.L., and K.B. analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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