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Different Pharmacokinetic and Pharmacodynamic Properties of Recombinant Follicle-Stimulating Hormone (rFSH) Derived From a Human Cell Line Compared With rFSH From a Non-Human Cell Line

Håkan Olsson, PhD, Rikard Sandström, PhD, and Lars Grundemar, MD, PhD

Abstract

Pharmacokinetic and pharmacodynamic properties of a novel recombinant follicle-stimulating hormone (rFSH) preparation (FE 999049), expressed by a human cell line (PER.C6), was compared with an rFSH preparation (follitropin α) expressed by a Chinese hamster ovary (CHO) cell line in healthy pituitary-suppressed women. Following single intravenous administration of 225 IU (Steelman–Pohley assay), the clearance was lower, 0.31 versus 0.44 L/h, for FE 999049 than for follitropin α. Likewise, the apparent clearance after repeated daily subcutaneous administrations was lower, 0.58 versus 0.99 L/h, and AUC and C_{max} higher, 1.7- and 1.6-fold. The absolute bioavailability after a single subcutaneous dose of 450 IU was similar for both preparations, 60–65%. After repeated subcutaneous administration the elimination half-life was approximately 30 and 24 hours for FE 999049 and follitropin α. The ovarian responses by number of follicles and serum concentrations of inhibin B and estradiol, were higher with FE 999049 than with follitropin α, AUC and C_{max} for the two latter being >1.6-fold greater with FE 999049 than with follitropin α. These results indicate that administration of equal doses of FE 999049, expressed in a human cell line, and follitropin α, expressed in a CHO cell line, display different pharmacokinetic and pharmacodynamic properties in humans.

Keywords

recombinant FSH, human cell line, pharmacokinetics, pharmacodynamics, bioavailability

Follicle-stimulating hormone (FSH) is produced in the anterior pituitary gland and is a key hormone in both male and female reproductive functions. In females, it stimulates growth and maturation of ovarian follicles[1] while in males it promotes spermatogenesis.[2] The synthesis and release of FSH is mainly controlled by the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus. Information of on the subsequent fate of FSH are limited, but animal data indicate that in addition to the ovaries, FSH is distributed mainly to the kidney and liver,[3,4] and predominantly eliminated by the kidney.[4–6]

FSH is a glycoprotein composed of 2 non-covalently bound polypeptide chains, denoted α and β. The α-subunit, which is common to pituitary FSH, pituitary luteinizing hormone (LH), pituitary thyroid-stimulating hormone (TSH), and placental chorionic gonadotropin (hCG), contains 92 amino acid residues, with 5 intra-disulfide bonds. The structurally different β-subunit, which is unique to FSH, contains 111 amino acid residues, with 6 intra-disulphide bonds. Both subunits of FSH are *N*-glycosylated at 2 positions, the carbohydrates constituting nearly one-third of the total mass, which give rise to numerous isoforms of the molecule with substantial variations in size and structure of the carbohydrate chains as well as the level of sialylation.[7,8] It has been demonstrated that the composition of the carbohydrate moieties has a significant impact on the in vivo activity of FSH by affecting the clearance, the binding properties to the FSH receptor in the target organs, and its ability to activate the receptors. Generally, less acidic isoforms have a higher clearance,[9–11] while the biological activity in vitro and in vivo has commonly been reported to be increased compared with isoforms with a higher number of sialic acid residues.[9,12–15]

Recombinant FSH (rFSH) has been widely used in the treatment of infertility since the introduction in the mid-1990s. To date, the currently available rFSH products

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used for controlled ovarian stimulation in women undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles, that is, follitropin α and follitropin β, are expressed in Chinese hamster ovary (CHO) cell lines.[16] Recently, a novel rFSH (FE 999049) derived from a cell line of human fetal retinal origin (PER.C6) entered clinical development. The amino acid sequences of the α- and β-subunits of FE 999049 are identical to the sequence of natural human FSH and existing CHO-derived rFSH products in clinical use, while the sialic acid content of FE 999049 is higher with both α2,3 and α2,6 sialylation and, thus, more resembling the natural human FSH than products from CHO cell lines with α2,3 sialylation only.[17]

This report presents results from two clinical studies comparing the pharmacokinetic and pharmacodynamic properties of FE 999049, derived from a human cell line, and follitropin α, derived from a CHO cell line, in healthy women of reproductive age. Since FE 999049 is the first rFSH derived from a human cell-line, a single dose intravenous (i.v.) and subcutaneous (s.c.) investigation was conducted to establish the true intrinsic pharmacokinetic properties, that is, clearance, distribution volume, and bioavailability, while the repeated s.c. part was undertaken to compare the pharmacokinetic and pharmacodynamic properties under more clinically relevant conditions.

Methods

Study Design

Each study protocol and informed consent document were reviewed and approved by Independent Investigational Review Board, Inc., Plantation, FL, USA. The studies were conducted in accordance with Declaration of Helsinki and the principles of Good Clinical Practice at Clinical Pharmacology of Miami, FL, USA. Prior written informed consent was obtained from all subjects.

The single dose pharmacokinetics and absolute bioavailability were assessed in an open-label, randomized, parallel, within group cross-over study in healthy women with suppressed endogenous FSH release. The subcutaneous administrations of FE 999049 and follitropin α were performed using different devices, and since the primary endpoints were based on objective measurements of FSH concentration in plasma, a double-dummy set-up for blinding was not considered justified. Subjects found eligible started a high-dose combined oral contraceptive (OGESTREL 0.5/50, Watson Pharma, Inc., Morristown, NJ, USA) run-in period 14 days before rFSH administration without any hormone-free period prior to the switch from the usual contraceptive. Daily administrations of combined oral contraceptive then continued throughout the study. The FSH level was analyzed before rFSH administration, and was required to be <5 IU/L for the subject to be eligible.

On the day of rFSH administration, each subject was randomized according to a computer generated randomization list with a block size of 10, provided by an independent statistician, to receive an i.v. dose, administered as a slow injection over 1–2 minutes, of either 225 IU FE 999049 or 225 IU follitropin α. Blood samples for pharmacokinetic analysis were collected pre-dose, and 10, 12, 15, 20, 30, 60, and 90 minutes, and 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 hours, and 3, 4, 5, and 6 days after administration. Two to five days after the last assessment of the i.v. administration, and after confirmation of suppressed endogenous FSH level, the subject received an abdominal s.c. injection of 450 IU of the same rFSH, FE 999049 or follitropin α, as was received in the first treatment period. Blood samples for pharmacokinetic analysis were collected pre-dose, and 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 hours, and 3, 4, 5, 7, 8, and 9 days after administration.

The repeated dose pharmacokinetic and pharmacodynamic investigation was a double-blind, randomized, parallel group study in healthy women. The endogenous FSH release was suppressed by a 1-month depot formulation of a GnRH agonist (LUPRON DEPOT, Abbott Laboratories, North Chicago, IL, USA) administered 28 and 14 days prior to the first administration of FE 999049/follitropin α. To confirm down-regulation of FSH and estradiol, serum levels were analyzed prior to the first rFSH administration and required to be <5 IU/L and ≤50 pg/mL, respectively, for the subject to be eligible. A cross-over design was not considered justifiable due to the lengthy need for continuous down-regulation of the healthy women with the GnRH agonist.

On the first day of rFSH administration, the subjects were randomized according to a computer generated randomization list with a block size of 4, provided by an independent statistician, to treatment with either FE 999049 or follitropin α. The subjects received a daily subcutaneous administration of 225 IU FE 999049 or 225 IU follitropin α for 7 days, each time at different abdominal locations and immediately after blood samples for pharmacokinetic (FSH) and pharmacodynamic (inhibin B and estradiol) assessments were collected. Following the last administration on Day 7, blood samples for pharmacokinetic and pharmacodynamic analyses were collected 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 hours after administration, and then daily up to 9 days after the administration.

Subjects

Healthy women, 21–35 years of age, were enrolled for the single dose pharmacokinetic and bioavailability investigation. The main inclusion criteria were body mass index (BMI) between 18 and 29 kg/m^2, menstrual cycle of 24–35 days, and the use of combined oral contraceptive for at least 3 months prior to screening. The same main

inclusion criteria applied for the repeated administration pharmacokinetic and pharmacodynamic investigation with the exception that women 21–39 years of age could participate. The slightly extended age range covered possible pharmacodynamic variability due to age in the target patient population.

Sources and Analysis of FE 999049 and Follitropin α

All references to FSH activity (IU) are based on the method by Steelman and Pohley.[18] FE 999049 was produced in a cell line of human fetal retinal origin (PER.C6) and provided as a sterile phosphate-buffered solution of pH 6–7. The concentration of the active ingredient was 600 IU/mL FSH activity, to be used undiluted for the s.c. administrations and diluted with saline for the i.v. injections. The commercial pack of a multidose pen follitropin α (GONAL-F, EMD Serono, Inc., Rockland, MA, USA) with a labeled content of 600 IU/mL follitropin α was used undiluted for the s.c. administrations and diluted with saline for the i.v. injections in the single-dose pharmacokinetic and bioavailability investigation. For the repeated dose pharmacokinetic and pharmacodynamic investigation, the commercially available freeze-dried powder in vials with a labeled content of 600 IU follitropin α was used together with a pre-filled syringe containing 1 mL solvent.

Analysis of serum concentrations of FSH were performed by the Department of Bioanalysis at Ferring Pharmaceuticals A/S by means of a validated immunoassay based on electrochemiluminescence (ECL). Briefly, a 96-well microplate was coated with a mouse anti-human FSH antibody (AbD Serotec, Oxford, UK) over night, after which samples were transferred by the aid of a Tecan Genesis Freedom sample handling robot. After adding the MSD-SULFO-TAG labeled mouse anti-human FSH antibody and incubating for 1 hour, the color was developed and read with an MSD Sector Imager 2400. The lower limits of quantification were 1.32 mIU/mL FE 999049 and 1.02 mIU/mL follitropin α, respectively, as determined from QC samples. The bias at all levels was within ±8%, and the bias for back-calculated calibration samples within 2%.

Pharmacodynamic Assessments

Follicle numbers and size were assessed by transvaginal ultrasonography, and blinded analysis of the digital images produced was performed by a gynecologist at the site. Inhibin B and estradiol were analyzed by Allied Research Laboratory (Miami, FL, USA) using commercially available immunoassay kits according to the manufacturer's instructions. Inhibin B was quantitated by the Inhibin B Gen II ELISA (Beckman Coulter, Inc., Brea, CA, USA), an enzymatically amplified 3-step sandwich assay. The lower limit of quantification was 4.8 pg/mL and the upper limit 500 pg/mL, assay performance %CV being within 3%. Estradiol was analyzed in the ADVIA Centaur System (Allied Research International, Miami Gardens, FL, USA), which is a competitive immunoassay using direct chemiluminescent technology. The lower limit of quantification of estradiol was 10 pg/mL and the upper limit 1000 pg/mL, assay performance % CV being within 1%.

Statistical Analyses

The pharmacokinetic parameters were calculated for baseline corrected data, assuming a constant background FSH concentration, by non-compartmental analysis (NCA) using the software WinNonlin® Pro (Pharsight Corporation, Cary, NC, USA). The baseline value was the mean of the values obtained prior to the first administration of rFSH. Serum concentration values below lower limit of quantification and missing values (eg, no blood sample collected or no value obtained at analysis) were excluded from the NCA. Actual time-points for blood sampling were used in the calculations of the individual parameters, while nominal time points were used for summary statistics. Pharmacokinetic parameters were estimated based on measurements from Day 1 to the last day of pharmacokinetic assessment, that is, Day 10 for the single administration and Day 16 for the repeated administration. The pharmacokinetic parameters at steady state for the repeated administration were estimated during one dosing interval, that is, 24 hours, after administration on Day 7.

Descriptive statistics were calculated for plasma concentrations and pharmacokinetic parameters, and all pharmacokinetic parameters were compared between FE 999049 and follitropin α for single i.v. and s.c., and repeated s.c. administrations using an ANOVA model for the log-transformed values. The ratios of the pharmacokinetic parameters were estimated with 90% confidence intervals. Serum estradiol and inhibin B concentrations were summarized by treatment group and measurement time, and the AUC_t and C_{max} estimates were compared across treatments using an ANOVA model for the log transformed values, with 90% confidence intervals. Number and size of follicles were summarized by size categories and treatment group and assessment time, and evaluated by descriptive statistics. The medians were compared between FE 999049 and follitropin α and evaluated by the Wilcoxon test. All statistical analyses were performed using SAS software version 9.2 for Windows (SAS Institute, Inc., Cary, NC, USA).

Results

Study Subjects

Fifty healthy women aged between 21 and 35 years were randomized and dosed for the single dose pharmacokinetic and bioavailability investigation, 25 in each treatment group. The body weight ranged from 49 to

85 kg and BMI ranged from 19.3 to 29.4 kg/m^2. The treatment groups were comparable with respect to demographic parameters (Table 1), as well as concomitant medication and medical history. Forty-eight of the 50 subjects completed both treatments while 2 subjects in the follitropin α treatment group withdrew consent due to personal reasons prior to completion of the study.

For the repeated dose pharmacokinetic and pharmacodynamic investigations 49 healthy women aged between 21 and 39 years were randomized in the study after completion of the run-in phase, 24 to receive FE 999049 and 25 to receive follitropin α. The body weight and BMI ranged from 46 to 88 kg, and from 19.4 to 28.9 kg/m^2, respectively. There were no relevant differences in demographic parameters (Table 1) or medical history and concomitant medication between the 2 treatment groups. Twenty-four subjects in each treatment group completed the study, while 1 subject was prematurely withdrawn due to urinary tract infection, an adverse event that was regarded by the investigator as unrelated to treatment.

Single Dose i.v. and s.c. Pharmacokinetics

The mean overall time courses for FSH concentration in serum after a single i.v. administration of 225 IU of FE 999049 and follitropin α showed similar appearances (Figure 1A). The serum FSH concentration decreased less rapidly after administration of FE 999049 than after administration of follitropin α, and was from 1 hour after administration higher at all time points. The clearance for FE 999049 and follitropin α was 0.31 and 0.44 L/h, respectively, and consequently, the mean AUC was 1.4-fold greater for FE 999049 compared with follitropin α (Table 2). The confidence intervals for the ratios indicated that AUC, AUC_t, and clearance could not be claimed to be equal, while this could not be excluded for the terminal half-life and the distribution volume (Table 2).

In accordance with the observations after i.v. administration, s.c. administration of a single dose of 450 IU of the two rFSH preparations resulted in 1.5-fold greater exposure (AUC), 1.3-fold higher C_{max}, and 1.2- and 1.3-fold longer half-life and t_{max}, respectively, for FE 999049 compared with follitropin α (Figure 1A, Table 2). Similar ANOVA comparisons as for the i.v. administration showed that the 90% confidence intervals for the ratios indicated that none of the parameters, possibly with the exception of t_{max}, could be claimed to be equal (Table 2).

The estimates of the absolute bioavailability after single dose s.c. administration of 450 IU of FE 999049 and follitropin α showed an approximate mean bioavailability of 60–65% (Table 2). The ratio between the two rFSH preparations, 1.06, and the 90% confidence interval for the ratio, [093; 122], did not indicate any difference in bioavailability.

Repeated s.c. Dose Pharmacokinetics

Following the administration of 225 IU/day of FE 999049 or follitropin α over 7 days, the FSH trough concentration increased and reached a steady-state level after 6–7 days for both preparations (Figure 1B). After administration of FE 999049, the mean serum concentration of FSH was at each time-point higher, and over time increasingly so, as compared with the serum FSH concentration following administration of follitropin α, and the mean AUC_τ and AUC_{inf}, as calculated after the last dose, were both greater for FE 999049 compared with follitropin α. The kinetic parameters AUC_τ, AUC_{inf}, and C_{max} were significantly higher for FE 999049 compared to follitropin α, AUC_τ and AUC_{inf}, being 1.6-fold ($P < 0.001$) and 1.7-fold ($P < 0.001$) greater, and the C_{max} being 1.6-fold ($P < 0.001$) higher (Table 3). The apparent clearance (ie, the ratio CL/F) was higher for follitropin α compared with FE 999049, arithmetic mean (SD) being 0.99 (0.41) versus 0.58 (0.13) L/h ($P < 0.001$), while the apparent distribution volumes (V_z/F) were comparable, 30.4 (13.3) versus 24.2 (6.7) L. In addition, the mean terminal half-life was longer for FE 999049, 29.8 (8.1) versus 24.2 (13.2) hours ($P < 0.02$), while the t_{max} occurred later, 10.3 (6.6) versus 7.3 (5.0) hours, although not statistically significantly, compared with follitropin α.

Repeated s.c. Dose Pharmacodynamics

Both the number and the size distribution of growing follicles changed during the treatment period of 7 days and the following discontinuation of dosing. Throughout the assessment period, the median numbers of follicles in all size categories tended to be higher with FE 999049

Table 1. Summary of Demographic Characteristics

	Single Dose i.v. and s.c. Within Group Cross-Over Administration		Repeated Dose s.c. Administration	
	FE 999049 (N = 25)	Follitropin α (N = 25)	FE 999049 (N = 24)	follitropin α (N = 25)
Age (Years)	27 (4)	29 (4)	31.1 (5.3)	33.4 (5.0)
Weight (kg)	67.5 (9.5)	64.9 (8.5)	71.5 (10.0)	64.7 (11.8)
BMI (kg/m^2)	25.9 (3.0)	24.9 (3.0)	26.6 (2.4)	25.0 (3.2)

Data are arithmetic mean (standard deviation). BMI, body mass index; N, number of subjects.

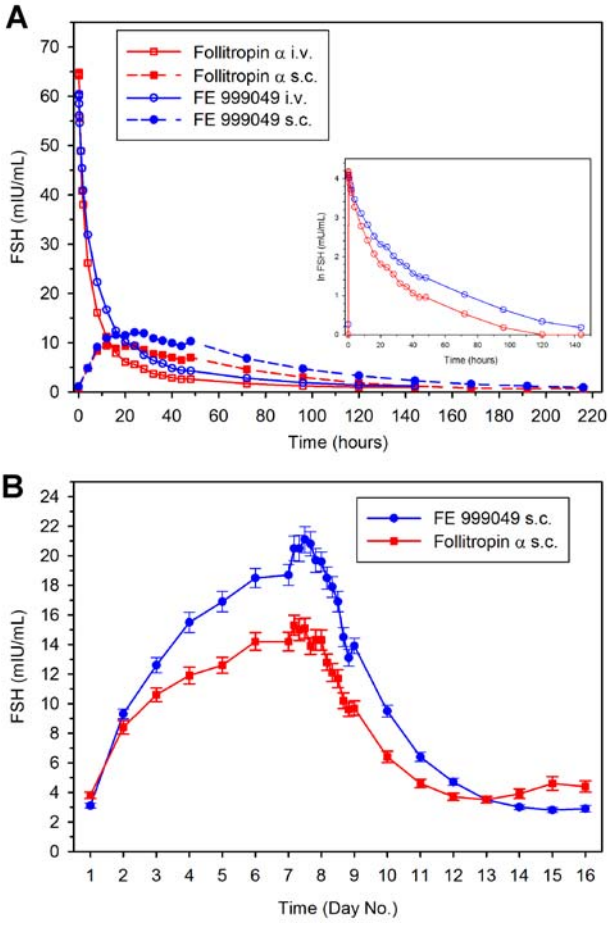

Figure 1. Time courses of serum FSH mean concentrations after administration of FE 999049 or follitropin α to healthy women. (A) Each woman received a single i.v. administration (225 IU) followed by a single s.c. administration (450 IU) in a cross-over design. The insert shows the i.v. time courses in a lin-log representation. Error bars are excluded in order to increase readability. (B) Each woman received s.c. administrations (225 IU) once daily for 7 consecutive days. Concentrations on Days 1–6 are pre-dose values. Bars represent standard error.

compared with follitropin α (Figure 2). The median total number of follicles continued to increase also after the end of treatment, and was significantly higher after treatment with FE 999049 on Day 11 (32 vs. 20, $P < 0.05$) and Day 15 (33 vs. 19, $P < 0.05$).

The serum concentrations of inhibin B and estradiol both increased subsequent to the increase in serum FSH concentration, however, with a slight time lag and to varying degrees (Figure 3). The mean serum concentration of inhibin B following administration of FE 999049 increased 90-fold, from a mean (SD) of 22 (23) pg/mL to a maximum of 2.0 (1.8) ng/mL on Day 7 and was maintained over the next 2 days, after which it declined. Follitropin α induced an inhibin B increase of similar general appearance, but with a maximum mean concentration of 1.3 (0.9) ng/mL on Day 8, a 47-fold increase from 27 (31) pg/mL. The mean serum concentration of estradiol with FE 999049 increased more than 70-fold from 24 (12) pg/mL to a maximum mean of 1.8 (1.7) ng/mL on Day 10. Follitropin α induced an estradiol increase of similar general appearance but at lower amplitude, with a maximum mean of 1.0 (1.0) ng/mL, a 40-fold increase from 25 (17) pg/mL. Both inhibin B and estradiol returned to baseline levels 8–10 days after the last administration. AUC and C_{max} for inhibin B and estradiol were significantly higher ($P < 0.05$) for FE 999049 compared with follitropin α (Table 3).

Safety

Both FE 999049 and follitropin α were well tolerated after s.c. as well as i.v. administration. The only adverse events occurring more than once and regarded as related to the treatment after repeated s.c. administration were headache, affecting 10 (42%) subjects in the FE 999049 group and 3 (12%) subjects in the follitropin α group, pelvic discomfort, affecting 3 (13%) subjects in the FE 999049 group, and edema and hot flush, affecting 2 (8%) subjects each in the follitropin α group. Adverse events regarded as related to the treatments were generally of mild intensity and did not cause any discontinuation, and there were no clinically significant findings in the clinical laboratory investigations, vital signs, or ECG.

Two subjects in the follitropin α group in the single dose part withdrew their consent due to personal reasons prior to completing the trial, one after the i.v. session and one 48 hours after the s.c. administration. In addition, 1 subject in the FE 999049 group in the repeated dose part was prematurely discontinued after the 4th dose due to urinary tract infection, an adverse event regarded by the investigator as not related to the treatment.

Discussion

This report is the first to demonstrate that the pharmacokinetic and pharmacodynamic properties of rFSH expressed in a human cell line differs from those of rFSH expressed in a CHO cell line, when administered to humans. The consistently higher exposure, longer time to C_{max}, and longer $t_{1/2}$ of FE 999049 after a single administration, and longer $t_{1/2}$ at steady state after repeated administrations, compared with follitropin α, indicated that either absorption or elimination, or both parameters of the two rFSH products are to some extent different. However, since the bioavailability of the products is comparable, and the clearance of follitropin α was similar to previously reported values,[19] the differences observed in the pharmacokinetic parameters after single i.v. as well as repeated s.c. administrations is well explained by the significantly higher clearance of follitropin α compared with that of FE 999049.

Table 2. Comparison of Pharmacokinetic Parameters of FE 999049 and Follitropin α After Single Dose i.v. and s.c. Administrations in Healthy Women

	Intravenous Administration			Subcutaneous Administration		
	Arithmetic mean (SD)			Arithmetic mean (SD)		
	FE 999049 (N = 25)	Follitropin α (N = 25)	GMR (90% CI)	FE 999049 (N = 25)	Follitropin α (N = 24)	GMR (90% CI)
AUC (mIU*h/mL)	760 (155)	544 (120)	1.41 (1.26; 1.57)	1064(312)	691 (214)	1.54 (1.32; 1.81)
AUC_t (mIU*h/mL)	698 (144)	499 (104)	1.40 (1.26; 1.56)	940 (302)	619 (204)	1.52 (1.29; 1.80)
C_{max} (mIU/mL)	61.8 (11.0)	66.9 (11.4)	0.92 (0.85; 1.00)	12.5 (4.5)	9.8 (3.3)	1.27 (1.07; 1.51)
t_{max} (h)				23.0 (8.7)	19.3 (8.3)	1.20 (1.00; 1.45)
$t_{1/2}$ (h)	29.6 (12.0)	25.2 (13.2)	1.24 (0.96; 1.61)	48.5 (30.4)	34.5 (7.7)	1.28 (1.06; 1.54)
Clearance (L/h)	0.31 (0.07)	0.44 (0.13)	0.71 (0.64; 0.79)			
V_{ss} (L)	9.02 (2.34)	10.03 (2.99)	0.91 (0.79; 1.04)			
F (%)				66 (16)	63 (20)	1.06 (0.93; 1.22)

SD, standard deviation; N, number of subjects.
GMR indicates geometric mean ratio (90% confidence interval). The ratio and confidence intervals are estimated using an ANOVA on ln (parameter), including treatment group as fixed effect.

Furthermore, the increased sialylation level of FE 999049[17] leads to more acidic isoforms compared with follitropin α, which, in accordance with earlier observations showing that FSH isoforms with increased sialic acid content are associated with decreased metabolic clearance,[9–11] would lead to a higher exposure.

Despite that several of the pharmacokinetic parameters after single dose administration were shown to be different between the rFSH preparations, the absolute bioavailability after a single s.c. administration of FE 999049 and follitropin α was similar, approximately 60–65%. This is within the same range as previously reported for rFSH,[20,21] and compares well to other therapeutic proteins being subcutaneously administered.[22,23] The absorption after s.c. administration, with a late t_{max}, reflects a rather slow absorption rate, presumably via the lymphatic vessels as indicated for other proteins of comparable size.[24,25] Furthermore, the longer half-life after s.c. administration compared with i.v. administration for both compounds indicates that the apparent elimination rate after s.c. administration is determined by the slow absorption rate, so called flip-flop kinetics.

On repeated administrations, the time to steady-state level of the pre-dose FSH concentration was similar for both FE 999049 and follitropin α. While the pharmacokinetic parameters for follitropin α were close to those reported for follitropin β,[26] another rFSH product also derived from CHO cells, the administration of FE 999049

Table 3. Comparison of Pharmacokinetic and Pharmacodynamic Parameters after Repeated s.c. Administrations in Healthy Women

	Arithmetic Mean (SD)			
Parameter	FE 999049 (N = 24)	Follitropin α (N = 24)	GMR (90% CI)	*P*-value
FSH AUCτ (mIU*h/mL)	410 (93)	262 (99)	1.63 (1.40; 1.90)	<0.001
FSH AUC_{inf} (mIU*h/mL)	1225 (397)	804 (469)	1.74 (1.37; 2.21)	<0.001
FSH C_{max} (mIU/mL)	19.2 (4.4)	12.4 (4.5)	1.60 (1.38; 1.86)	<0.001
FSH CL/F (L/h)	0.58 (0.13)	0.99 (0.41)	0.61 (0.53; 0.71)	<0.001
FSH V_z/F (L)	24.2 (6.7)	30.4 (13.3)	0.84 (0.71; 1.00)	0.101
FSH t_{max} (h)	10.3 (6.6)	7.3 (5.0)	1.37 (1.03; 1.83)	0.074
FSH $t_{1/2}$ (h)	29.8 (8.1)	24.2 (13.2)	1.38 (1.11; 1.70)	0.016
Inhibin-B AUC_{inf} (ng/mL days)	12.6 (11.1)	6.33 (4.07)	1.83 (1.24; 2.71)	0.013
Inhibin-B C_{max} (ng/mL)	2.34 (1.93)	1.34 (0.97)	1.67 (1.13; 2.47)	0.033
Estradiol AUC_{inf} (ng/mL days)	9.55 (11.5)	4.17 (4.78)	2.26 (1.30; 3.92)	0.017
Estradiol C_{max} (ng/mL)	2.03 (2.18)	1.02 (1.12)	2.10 (1.24; 3.57)	0.023

N, number of subjects; SD, standard deviation; AUCτ, AUC during one dosing interval, that is, 24 hours.
Each woman received a daily subcutaneous administration of either FE 999049 or follitropin α for 7 consecutive days. All pharmacokinetic and pharmacodynamic parameters are calculated from plasma concentrations following the last administration. Endpoints are ln-transformed before analysis, and results are transformed back and presented as ratios. The model is an ANOVA and includes treatment as fixed effect. GMR indicates geometric mean ratio (90% confidence interval). The mean is the geometric least squares means estimated from the model.

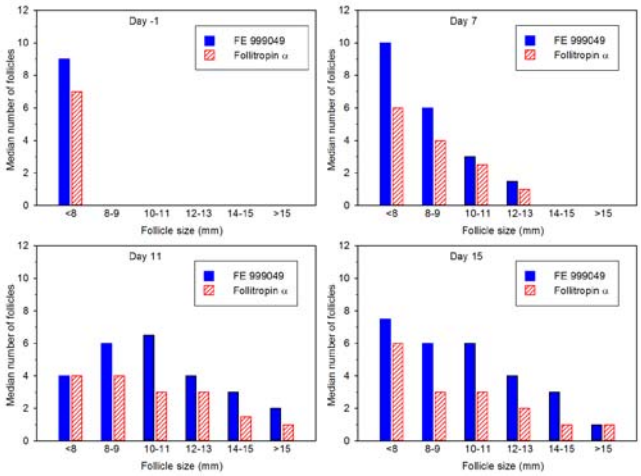

Figure 2. Median number of follicles in different size categories after repeated s.c. administrations of 225 IU FE 999049 or follitropin α to healthy women once daily for 7 consecutive days.

resulted in higher C_{max} and longer $t_{1/2}$, translating into a substantially greater exposure than follitropin α, both during the whole treatment period as well as during a dosing interval. Thus, it seems that different rFSH preparations of common origin possess similar properties, while different origins may cause discrepancies in the pharmacokinetic profile.

An interesting observation, common for both types of rFSH compounds, was that the clearance seemed to be higher and the terminal half-life to be shorter after 1 week of s.c. administrations compared with single administration. The mechanism behind this phenomenon is not known, but it has been observed previously with CHO-derived rFSH[17] as well as menotropin preparations (MENOPUR; unpublished data).

The dosing period of 7 days in the present study was somewhat shorter than the normal stimulation period of around 9–10 days in women undergoing IVF treatment. The time-course profiles of the increases of serum inhibin B and estradiol were very similar for both FE 999049 and follitropin α, and corresponded to the increase in FSH concentration. Seven days of subcutaneous treatment was sufficient to reach pharmacokinetic steady state, while, as previously observed with rFSH,[27] the pharmacodynamic responses initially lagged behind but tended to continue to grow also a few days after the last treatment. Since neither inhibin B nor estradiol appeared to have reached steady state after 7 days of treatment, it is not unlikely that continued treatment with rFSH would have resulted in further increased concentrations. The magnitudes of the

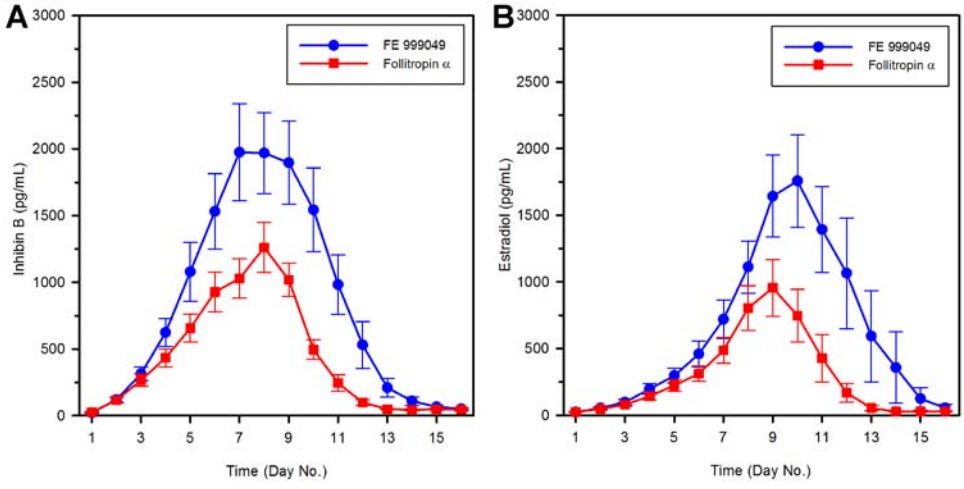

Figure 3. Time courses of inhibin B (A) and estradiol (B) mean (SE) concentrations in serum after repeated s.c. administrations of 225 IU FE 999049 or follitropin α once daily for 7 consecutive days. Concentrations on Days 1–7 are pre-dose values.

hormone responses were consistently higher following administration of FE 999049 compared with follitropin α and were in good agreement with the number and size of growing follicles. Together these findings demonstrate the differences in pharmacodynamic properties between rFSH from the human cell line and from a CHO cell line.

The quantification of FSH bioactivity of both FE 999049 and follitropin α was performed using the Steelman–Pohley in vivo rat assay.[18] Based on the results in the present investigation it is clear that the same bioactivity in rats of rFSH, expressed in a human cell line or a CHO cell line, will not translate into the same bioactivity for the 2 rFSH products in humans.

In conclusion, the results of the present investigation clearly indicate that the preparations of rFSH from a human cell line and a CHO cell line display significant differences in pharmacokinetic and pharmacodynamic properties. The exact mechanism remains to be elucidated, but since the amino acid sequences are identical it seems likely that the more acidic properties of FE 999049, thereby decreasing the clearance, cause greater exposure and higher biopotency in terms of increased follicular growth with this human-derived rFSH preparation compared with CHO-derived follitropin α. However, to predict to what extent the different properties of these rFSH products translates into clinical practice and outcome would at this stage only be speculative. This needs to be more accurately addressed in a dedicated clinical study in patients assessing ovarian response, ongoing pregnancy rate, and other clinically related parameters.

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Declaration of Conflicting Interests

Håkan Olsson, Rikard Sandström, and Lars Grundemar are all employees of Ferring Pharmaceuticals A/S.

References

1. Hillier SG. Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. *Hum Reprod*. 1994;9:188–191.
2. Simoni M, Weinbauer GF, Gromoll J, Nieschlag E. Role of FSH in male gonadal function. *Ann Endocrinol (Paris)*. 1999;60:102–106.
3. Butt WR, Ryle M, Shirley A. Kinetic studies with 125I-labelled follicle-stimulating hormone. *J Endocrinol*. 1973;58:275–287.
4. Sebok K, Sairam MR, Cantin M, Mohapatra SK. Distribution of follitropin and deglycosylated follitropin in the rat: a quantitative and radioautographic study. *Mol Cell Endocrinol*. 1987;52:185–197.
5. Emmanouel DS, Stavropolous T, Katz AI. Role of the kidney in metbolism of gonadotropins in rats. *Am J Physiol*. 1984;247:E786–E792.
6. Nisula BC, Blithe DL, Akar A, Lefort G, Wehmann RE. Metabolic fate of human choriogonadotropin. *J Steroid Beiochem*. 1989:733–737.
7. Pierce JG, Parsons TF. Glycoprotein hormones: structure and function. *Annu Rev Biochem*. 1981;50:465–495.
8. Ulloa-Aguirre A, Timossi C, Damián-Matsumura P, Dias JA. Role of glycosylation in function of follicle-stimulating hormone. *Endocrine*. 1999;11:205–215.
9. Ulloa-Aguirre A, Timossi C, Barrios-de-Tomasi J, Maldonado A, Nayudu P. Impact of carbohydrate heterogeneity in function of follicle-stimulating hormone: studies derived from in vitro and in vivo models. *Biol Reprod*. 2003;69:379–389.
10. Goochee CF, Gramer MJ, Anderson DC, Bahr JB. Rasmunsen JR The oligosaccharides of glycoproteins: bioprocess factors affecting oligosaccharide structure and their effect on glycoprotein properties. *Biotechnology*. 1991;9:1347–1355.
11. D'Antonio M, Borrelli F, Datola A, et al. Biological characterization of recombinant human follicle stimulating hormone isoforms. *Human Reprod*. 1999;14:1160–1167.
12. Zambrano E, Zarinan T, Olivares A, Barrios-de-Tomasi J, UlloaAguirre A. Receptor binding activity and in vitro biological activity of the human FSH charge isoforms as disclosed by heterologous and homologous assay systems: implications for the structure-function relationship of the FSH variants. *Endocrine*. 1999;10:113–121.
13. Barrios-De-Tomasi J, Timossi C, Merchant H, et al. Assessment of the in vitro and in vivo biological activities of the human follicle-stimulating isohormones. *Mol Cell Endcrinol*. 2002;186:189–198.
14. Loreti N, Fresno C, Barrera D, et al. The glycan structure in recombinant human FSH affects endocrine activity and global gene expression in human granulosa cells. *Mol Cell Endocrinol*. 2013;366:68–80.
15. de Leeuw R, Mulders J, Voortman G, Rombout F, Damm J, Kloosterboer L. Structure-function relationship of recombinant follicle stimulating hormone (Puregon). *Mol Hum Reprod*. 1996;2:361–369.
16. Howles C. Genetic engineering of human FSH (Gonal-F). *Hum Reprod Update*. 1996;2:172–191.
17. World Intellectual Property Organisation (WO 2009/127826 Al) Recombinant FSH including α 2,3 and α 2,6 sialylation.
18. Steelman SL, Pohley FM. Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotropin. *Endocrinology*. 1953;53:604–616.
19. le Cotonnec J-Y, Porchet HC, Beltrami V, Kahn A, Toon Rowland SM. Clinical pharmacology of recombinant human follicle-stimulating hormone (FSH): part I. Comparative pharmacokinetics with urinary human FSH. *Fertil Steril*. 1994;61:669–678.
20. Loumaye E, Campbell R, Salat-Baroux J. Human follicle-stimulating hormone produced by recombinant DNA technology: a review for clinicians. *Hum Reprod Update*. 1995;1:188–199.
21. le Cotonnec J-Y, Porchet HC, Beltrami V, Kahn A, Toon S, Rowland M. Clinical pharmacology of recombinant human follicle-stimulating hormone: part II. Single dose and steady state pharmacokinetics. *Fertil Steril*. 1994;61:679–686.
22. Trinchar-Lugan I, Khan A, Porchet HC, Munafo A. Pharmacokinetics and pharmacodynamics of recombinant human chorionic gonadotrophin in healthy male and female volunteers. *Reprod Biomed Online*. 2002;4:106–115.
23. le Cotonnec J-Y, Porchet HC, Beltrami V, Munafo A. Clinical pharmacology of recombinant human luteinizing hormone: part II. Bioavailability of recombinant human luteinizing hormone assessed with an immunoassay and an in vitro bioassay. *Fertil Steril*. 1998;69:195–200.
24. Porter CJ, Charman SA. Lymphatic transport of proteins after subcutaneous administration. *J Pharm Sci*. 2000;89:297–310.

25. Ballard BE. Biopharmaceutical considerations in subcutaneous and intramuscular drug administration. *J Pharm Sci*. 1968;57: 357–378.
26. Voortman G, Mannaerts BMJL, Huisman JAM. A dose proportionality study of subcutaneously and intramuscularly administered recombinant human folliclestimulating hormone (Follistim/Puregon) in healthy female volunteers. *Fertil Steril*. 2000;73 (6):1187–1193.
27. Porchet HC, le Cotonnec J-Y, Loumaye E. Clinical pharmacology of recombinant human follicle-stimulating hormone. III. Pharmacokinetic-pharmacodynamic modeling after repeated subcutaneous administration. *Fertil Steril*. 1994;61:687–695.