Single-dose pharmacokinetics and pharmacodynamics of recombinant human follicle-stimulating hormone (Org 32489*) in gonadotropin-deficient volunteers[††]

Bernadette Mannaerts, M.Sc.,§ Zeev Shoham, M.D.,‖¶ Dick Schoot, M.D.,††
Philippe Bouchard, Ph.D.,‡‡ Jonas Harlin, M.D.,§§ Bart Fauser, Ph.D.,††
Howard Jacobs, M.D.,‖ Ferdy Rombout, M.Sc.§ and Herjan J. T. Coelingh Bennink, M.D., Ph.D§

Organon International, Oss, the Netherlands; Middlesex Hospital, London, United Kingdom; Dijkzigt University Hospital, Rotterdam, the Netherlands; Hôpital Bicêtre, Le Kremlin Bicêtre, France; Karolinska Hospital, Stockholm, Sweden

Objective: To assess safety, pharmacokinetic, and pharmacodynamic properties of recombinant human follicle-stimulating hormone (FSH; Org 32489, Organon International, Oss, the Netherlands) after a single intramuscular injection in the buttock.

Design: In a prospective study, safety variables, serum FSH, luteinizing hormone, inhibin, estradiol (females only), and testosterone (males only) were evaluated up to a maximum of 11 days after injection of 300 IU recombinant FSH.

Setting: Four specialist Reproductive Endocrinology and Infertility units.

Volunteers: Fifteen men and women exhibiting all pituitary gonadotropin deficiency.

Result(s): A single bolus of 300 IU recombinant FSH was well tolerated, and no drug-related adverse effects were noted. Comparison of before and after treatment safety variables, including serum antirecombinant FSH antibodies, showed no changes of clinical relevance. Analysis of serum FSH levels revealed comparable elimination half-lives of 44 ± 14 (mean $\pm$ SD) and 32 ± 12 hours in women and men volunteers, respectively. In contrast, peak FSH concentrations were significantly lower in women than in men volunteers (4.3 ± 1.7 versus 7.4 ± 2.8 IU/L), and the time required to reach peak levels of FSH was significantly longer in women than in men (27 ± 5 versus 14 ± 8 hours). The area under the serum level versus time curve tended to be smaller in women than in men volunteers (339 ± 105 versus 452 ± 183 IU/L $\times$ hours), but the difference did not reach statistical significance. Together these data suggest that recombinant FSH is absorbed from its intramuscular depot to a lower rate and extent in women than in men. In both sexes a relationship between serum FSH levels and body weight was apparent. During the experimental period, other hormones remained low at baseline levels or were only slightly increased.

Conclusion(s): Our findings indicate that recombinant FSH is well tolerated and that it is absorbed from its intramuscular depot to a higher rate and extent in men than in women. After intramuscular administration, its half-life is in good agreement with that previously reported for natural FSH. (Fertil Steril® 1993;59:108–14. ©1993 by American Society for Reproductive Medicine.)

Key Words: Recombinant human FSH, single-dose pharmacokinetics

Human follicle-stimulating hormone (FSH) is a gonadotropic hormone produced by the anterior pituitary gland, whose primary function is regulation of follicular growth in females and of spermatogenesis in males. Hormonal response is accomplished via specific membrane receptors on granulosa cells and Sertoli cells, causing adenylate cyclase activation and thereby secretion and/or synthesis of various factors essential for target cell differentiation and gamete maturation (1, 2).

The FSH molecule has a dimeric structure of which both subunits are glycoproteins in nature. The 92-amino acid α-chain and the 111-amino acid β-chain have each two N-linked oligosaccharide chains presented as complex heterogeneous multiantennary structures (3). The variable degree of glycosylation, especially of sialylation, creates a spectrum of FSH isoforms with differences in charge, bioactivities, and elimination half-lives (4, 5).

The expression of human FSH in Chinese hamster ovary cells transfected with both subunit genes (6, 7) resulted in the synthesis of intact human FSH (recombinant FSH). The polypeptide backbone of recombinant FSH is identical to that of natural FSH, whereas recombinant and natural carbohydrate structures are closely related (8). The charge heterogeneity and bioactivity of recombinant FSH were confirmed by chromatofocusing, receptor displacement, and by in vitro and in vivo animal studies (9–11). In comparison with commercially available urinary gonadotropin preparations, highly pure (99.9%) recombinant FSH appeared to lack intrinsic luteinizing hormone (LH) activity and to exhibit a very high specific bioactivity (>10,000 IU/mg protein). These properties prompted the further development of recombinant FSH by means of clinical studies examining its pharmacokinetic and antigenic properties in humans. This first human exposure study was performed in gonadotropin-deficient male and female volunteers to assess the safety and tolerance and the pharmacokinetic and pharmacodynamic properties of recombinant FSH after a single intramuscular injection.

MATERIALS AND METHODS

Subjects and Study Design

Fifteen gonadotropin-deficient, but otherwise healthy, volunteers (8 women and 7 men) participated in this four-center study. The study protocol was approved by the local ethics review committees, and written informed consent was obtained from all volunteers. Nine subjects had panhypopituitarism, either primary ($n = 3$) or secondary ($n = 6$), due to surgical removal of a nonmalignant pituitary tumor. Five volunteers suffered from congenital isolated gonadotropin deficiency, and one volunteer was diagnosed as weight-loss–related hypothalamic hypogonadism. Autoimmunity was excluded by antinuclear and specific antirecombinant FSH antibody assays. Subjects receiving estrogen/androgen replacement refrained from this therapy, which started 1 week (oral therapy) or 3 weeks (intramuscular substitution) before injection up to 1 week after injection while appropriate thyroid and glucocorticoid therapy was continued. With the exception of one male volunteer, all subjects had a history of proven normal gonadal function; seven out of eight women had one or more deliveries, and one woman and six men subjects responded well to previous hormonal therapy.

Subjects received a single intramuscular injection of 300 IU recombinant FSH (Org 32489, CP 90073; Organon International, Oss, the Netherlands) in 2 mL solvent in the upper quadrant of the buttock. Blood samples were taken just before injection and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16 (optional), 24, 48, 72, 96, 120 (optional), 168, 216 (optional), and 264 hours (11 days) after injection. Blood samples were centrifuged, and serum was stored in 0.5-mL serovials at $-20°C$ until analysis. Serum was assayed for

immunoactive FSH, immunoactive and bioactive LH, testosterone (T), estradiol (E_2), and inhibin.

Safety Parameters

Safety analysis included clinical observations, i.e., blood pressure, heart rate, and body temperature, as well as laboratory assessments like routine urinalysis (pH, protein, acetone, glucose, hemoglobin), blood biochemistry (sodium, potassium, chloride, bicarbonate, phosphorus, calcium, glucose, urea, creatinin, uric acid, alkaline phosphatase, alanine and aspartate aminotransferase, lactic dehydrogenase, bilirubin, protein, albumin), and hematology (hemoglobin, hematocrit, erythrocytes, differentiated leucocytes, thrombocytes).

Serum samples were analyzed for the presence of antirecombinant FSH antibodies using a sensitive radioimmunoprecipitation assay and ^{125}I-recombinant FSH as a tracer. When testing a mixture of two mouse monoclonal antibodies (MCAs) raised against recombinant FSH and recognizing an α- and β-specific epitopes, the sensitivity of the assay was 0.5 pmol/L and the intra-assay and interassay coefficients of variation (CV) ranged from 4.3% to 9.6% and from 0.8% to 2.7%, respectively. The induction of antirecombinant FSH antibodies after recombinant FSH treatment was judged by comparing before and after treatment samples according to criteria, allowing a probability of a false-positive result of <0.1%. All serum samples were tested in duplicate, and the MCA mixture was used as a positive control in all experiments.

Hormone Assays

Immunoreactive FSH and LH was measured by an immunofluorometric assay using the time-resolved fluoroimmunoassay technique and reagent kits 1244-017 for human FSH and 1244-31 for human LH (Delfia, Pharmacia, Woerden, the Netherlands). These two-site assays use a β-directed capturing MCA and an α-directed europium-labeled detection MCA. The assays were performed as described by the manufacturer using the Delfia instrumentation system and MultiCalc software (Pharmacia). Follicle-stimulating hormone and LH immunoreactivity was expressed in terms of the 2nd International Reference Preparation (IRP) of pituitary FSH (code no. 78/549) and the 2nd International Standard for pituitary LH (code no. 80/552). The sensitivity of immunofluorometric assay was 0.05 IU/L for both gonadotropins, and the intra-assay and interassay CV were below 4.8% and 4.3% for FSH and 4.7% and 7.5% for LH, respectively. The cross-reactivity of the FSH kit with LH was <0.08% and of the LH kit with FSH <0.01%.

Serum bioactive LH was measured in an in vitro mouse Leydig cell bioassay as described in detail previously (10). The sensitivity of this assay for serum samples using 2nd International Standard 80/552 as the standard was 2 IU/L.

Serum T and E_2 were assessed by radioimmunoassay (RIA) using a coat-a-count T RIA (reagent kit TKTT1 DPC, detection limit 0.27 nmol/L; Diagnostic Products Corpora-

Clinical characteristics of volunteers.*

	Age	Weight	Height	BMI	FSH	LH
	y	*kg*	*cm*	*kg/m²*	*IU/L*	*IU/L*
Females ($n = 8$)	36 ± 3	67 ± 13	162 ± 12	26.1 ± 4.3†	0.54 ± 0.34	<0.17
Males ($n = 7$)	31 ± 7	62 ± 11	171 ± 13	21.3 ± 1.7	0.63 ± 0.57	<0.36

* Values are means ± SD.
† Significantly higher in females than in males.

tion, Los Angeles, CA) and a double antibody E_2 RIA (reagent kit KE2D1 DPC, detection limit 11.6 pmol/L; Diagnostic Products Corporation). The intra-assay and interassay CV were <9% and 13% for the T assay and <4% and 5% for the E_2 assay, respectively.

Serum inhibin levels were measured by RIA using an antiserum (no. 1989) raised against purified bovine 31-kd inhibin (12). Purified bovine 31-kd inhibin iodinated by the lactoperoxidase method was used as a tracer. The standard was a pool of human follicular fluid (FF; 280 U/mL) that was calibrated against a rete testis standard preparation of defined bioactivity. The immunoactivity of 1 mU FF was equipotent of 0.121 pg recombinant human inhibin (Biotech Australia, specific in vitro bioactivity 51.060 U/µg protein using World Health Organization [WHO] standard 86/690 as the standard). The recombinant α-subunit of human inhibin exhibited complete cross-reactivity in this assay system. The standard pool, which was diluted in plasma from castrated subjects, provided dose responses parallel to the plasma dilution curves. The sensitivity of the assay was 28 U/L and the intra-assay and interassay CV were <10%.

Data Analysis

The peak recombinant FSH concentration (C_{max}) and the time of its occurrence (T_{max}) were taken from measured serum level data. The area under the serum level versus time curve (AUC) after a single dose of 300 IU was determined by means of the trapezoidal rule from zero time up to infinity ($AUC_{0-\infty}$) under subtraction of the baseline. The elimination half-life ($t_{1/2}$) was calculated after baseline correction on the basis of increases of FSH concentrations measured between 72 and 264 hours after injection, using log-linear regression. Data are presented as mean ± SD unless stated otherwise. Curvefit coefficients (r) represent Pearson correlation coefficients. Comparison of age, height, weight, and body mass index (BMI) between male and female volunteers was performed by means of the Wilcoxon's test. Gender differences of bioequivalence were tested in a one-way analysis of variance (ANOVA). Differences were considered to be statistically significant if $P \leq 0.05$.

RESULTS

Volunteers

The mean age, weight, height, BMI, and serum gonadotropin levels at screening are listed in Table 1. No significant differences between male and female volunteers with respect to age, height, or weight were found, whereas BMI values were higher ($P = 0.04$) for the female volunteers. During screening, serum FSH levels ranged between 0.12 and 1.08 IU/L in males and between 0.11 and 1.63 IU/L in females. Serum LH levels were either undetectable (<0.05 IU/L) or very low, resulting in individual levels between <0.05 and 0.40 IU/L in females and between <0.05 and 1.13 IU/L in males.

Safety and Tolerance

A single injection of 300 IU recombinant FSH was well tolerated, and no drug-related adverse experiences were noted. Neither pain nor skin redness was observed at the site of drug injection. Comparison of before and after treatment safety variables, i.e., blood pressure, heart rate, body temperature, blood biochemistry, hematology, and urinalysis, revealed no changes of clinical significance. When screening volunteers for the possible induction of antirecombinant FSH antibodies, no post-treatment increases in ^{125}I-FSH binding were observed.

Pharmacokinetic Analysis

Individual and mean delta increases of serum FSH after intramuscular injection of 300 IU recombinant FSH in seven male and eight female volunteers are shown in Figure 1. In all subjects, serum FSH levels were raised at 30 minutes after injection, and 13 out of 15 volunteers returned to baseline values 264 hours after injection. Individual pharmacokinetic parameters, AUC, C_{max}, T_{max}, and $t_{1/2}$, are presented in Table 2. Within the group of male volunteers, one statistical outlier was identified by means of the Dixon test. This subject (M2), with an extremely low body weight (42 kg) and extremely high AUC value (876 IU/L × hours), was not included in mean values and was excluded from further statistical analysis. The mean $t_{1/2}$ of recombinant FSH was not significantly different between sexes (44 ± 14 versus 32 ± 12 hours). In contrast, C_{max} values were significantly

Individual and mean delta increases of serum immunoreactive FSH after intramuscular injection of 300 IU recombinant FSH in seven men (**A**) and eight women (**B**) with hypogonadotropic hypogonadism.

FSH (IU/L)
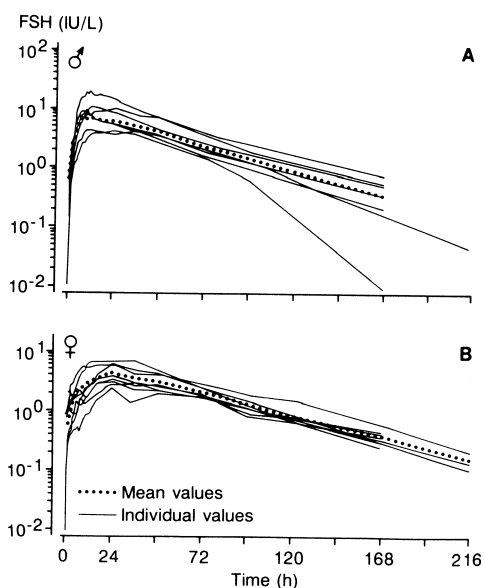
(*P* = 0.0072) lower in female than in male volunteers (4.3 ± 1.7 versus 7.4 ± 2.8 IU/L) and T_{max} was also significantly (*P* = 0.0004) longer in females than in males (27 ± 5 versus 14 ± 8 hours). The mean AUC after administration of 300 IU recombinant FSH was 339 ± 105 IU/L × hours in females and 452 ± 183 IU/L × hours in males and thus tended to be lower in females, although the difference was not statistically significant (*P* = 0.058).

Relationship Between Body Weight and Serum FSH Levels

Comparison of body weight and serum FSH levels revealed a negative relationship in both men and women. Although the number of subjects studied are limited, the data suggest that there is a linear relationship between body weight and C_{max} values (males *r* = 0.85; females *r* = 0.83) and between body weight and AUC values (males *r* = 0.89; females *r* = 0.86). Scatter plots illustrating these associations are presented in Figure 2. The apparent linear relationships between BMI and C_{max} values (males *r* = 0.48; females *r* = 0.61) and between BMI and AUC values (males *r* = 0.51; females *r* = 0.65) were less strong (data not shown).

Other Hormones

Serum LH levels were assessed during screening (see Table 1), just before injection, and at 1 and 3 days thereafter. Mean immunoreactive before and after treatment LH levels were below 0.4 IU/L for all subjects. Serum samples of five

female and five male volunteers were also tested in the in vitro LH bioassay, but serum bioactive LH was below the detection limit of the assay (<2 IU/L) in all cases.

In female volunteers, E_2 was detectable (>9.9 pmol/L) in only three women; 2 days after injection, two women showed a slight increase in E_2 (24 and 33 pmol/L, respectively). Serum inhibin was either undetectable (<30 U/L) or very low in female volunteers, whereas six out of seven males had detectable levels of inhibin. In comparison with baseline values, the mean inhibin of these six men was doubled (238 ± 91 versus 124 ± 66 U/L) 3 days after recombinant FSH injection. Serum T was either undetectable (<0.27 nmol/L) in two men or low (<10 nmol/L) in others, and no changes of any significance were noted (data not shown).

DISCUSSION

For nearly 30 years, infertility treatment with gonadotropins has been based on the application of crude urinary gonadotropin preparations, which have been proven safe and effective. The future of gonadotropin therapy, however, is likely to lie with highly pure recombinant human FSH preparations, devoid of other gonadotropins or inactive contaminants. Furthermore, FSH production by means of recombinant DNA technology is thought to guarantee an improved batch to batch consistency.

Individual and mean pharmacokinetic parameters of recombinant FSH after one single intramuscular injection in the gluteal area in females (F) and males (M).

|	AUC	C_{max}	T_{max}	$t_{1/2}$
	IU/L × hours	IU/L/h	h	h
F1	506	6.9	36	43
F2	255	2.8	35	35
F3	244	2.4	24	69
F4	252	3.1	24	40
F5	287	3.4	24	63
F6	292	3.8	24	33
F7	454	6.1	24	37
F8	425	5.9	24	31
Mean ± SD	399 ± 105	4.3 ± 1.7*	27 ± 5†	44 ± 14
M1	744	10.0	24	42
M2‡	876	18.4	10	40
M3	367	8.1	9	34
M4	599	10.9	10	44
M5	355	4.4	9	38
M6	250	4.1	24	12
M7	393	6.9	10	25
Mean ± SD	452 ± 183	7.4 ± 2.8	14 ± 8	32 ± 12

* Significantly lower in females than in males.
† Significantly longer in females than in males.
‡ Statistical outlier not included in mean values.

Correlation of C_{max} (**A**) and AUC (**B**) with body weight in seven men and eight women with hypogonadotropic hypogonadism.
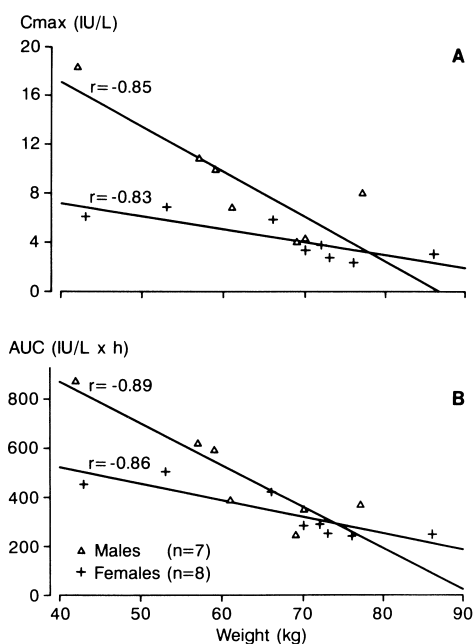
A general concern of recombinant glycoproteins is their potential immunogenicity. Because the peptide backbone structures of the natural and recombinant FSH were known to be identical, this concern was limited to possible minor differences in tertiary structure due to host cell processing. This first clinical study with recombinant FSH was performed in gonadotropin-deficient volunteers to minimize possible hazards in case of an antirecombinant FSH immune response and to prevent interference with endogenous gonadotropins. To date, many patients have been treated successfully with other recombinant glycoproteins, like erythropoietin, without developing specific antibodies (13). In the present study, no serum antirecombinant FSH antibodies were detected, but further studies will be required to demonstrate the safety of recombinant FSH during repeated administrations and long-term infertility therapy.

After intramuscular injection, the release rate of gonadotropins may depend on the formulation, injection depth, site, and volume (14). After intramuscular injection in the buttock, the absorption of immunoreactive recombinant FSH was very slow and even significantly slower in women than in men. Analysis of serum samples taken up to 72 hours after injection revealed that immunoreactive FSH levels were in good agreement with circulating bioactive FSH measured by an in vitro granulosa cell bioassay (Huhtaniemi I, personal communication).

Pharmacokinetic studies with urinary FSH and human

menopausal gonadotropin (hMG) preparations administered via the intramuscular route have been limited but demonstrated previously that serum FSH levels depend on both the absorption and excretion rate of the drug. Diczfalusy and Harlin (15) reported that $t_{1/2}$ of hMG after intramuscular administration ($>$40 hours) is about four times longer than after intravenous injection. Daily injection of 150 or 225 IU urinary FSH in three women with isolated gonadotropin deficiency revealed a mean $t_{1/2}$ of 36 $\pm$ 16 hours, whereas $t_{1/2}$ varied between 33 and 59 hours in normal men after single intramuscular administration (16). The $t_{1/2}$ of recombinant FSH in the present study seems to be in good agreement with those reported for urinary FSH/hMG, although this is the first report on different release rates of FSH in men and women. Pharmacokinetic parameters like T_{max} and C_{max} are defined by the release of the drug from the intramuscular depot and by its $t_{1/2}$. However, the latter was not significantly different between the sexes; seeming differences may be attributed to the large intersubject variability. Sex differences in drug absorption and bioavailability after injection of aqueous solutions in the gluteus maximus, rather than in the vastus lateralus or deltoid, have been described previously and are thought to be related to the gluteal fat thickness, which is known to be greater in women than in men (14). Consequently, women may receive part of the drug in their subcutaneous adipose layer rather than intramuscular, leading to a less rapid absorption. Whether the latter also results in a lower absolute bioavailability, as indicated by the relative small mean AUC of the female volunteers, remains to be assessed.

Various studies support the hypothesis that body weight is a major determining factor on the dose and length of gonadotropin stimulation for induction of ovulation and for in vitro fertilization (17, 18). Follicle-stimulating hormone doses to initiate ovarian response may vary largely between individuals (19), and also thereafter major differences in ovarian response require close treatment monitoring. The present study revealed a strong negative correlation between body weight and serum FSH levels after recombinant FSH administration, thus suggesting that adjustment of doses of FSH in relation to body weight could reduce ovarian response variability.

In the current study, all volunteers, one man excepted, had previous proof of normal gonadal function. In view of its slow disappearance rate after intramuscular injection, a single injection of recombinant FSH might have been sufficient to induce temporarily gonadal response, influencing directly or indirectly the synthesis of other hormonal factors. During the experimental period, circulating immunoactive and bioactive LH levels were extremely low or undetectable, as previously reported for patients with hypogonadotropic hypogonadism (20). Accordingly, serum T and E_2 levels were very low in male and female volunteers, respectively, and only two out of eight women showed a very small E_2 rise 2

days after injection. Follicle-stimulating hormone-induced E_2 synthesis, however, is known to be impaired in hypogonadotropic subjects (21–23), most likely because the minute amounts of residual LH are too low to support E_2 biosynthesis adequately. Interestingly, six out of seven men volunteers showed slightly increased serum inhibin levels 3 days after recombinant FSH injection. Using antiserum against bovine 31-kd inhibin, comparable levels of inhibin in men with hypogonadotropic hypogonadism were reported by others (24). The fact that no inhibin increases were observed in the female volunteers might be related to the relatively lower availability of recombinant FSH in these subjects.

In conclusion, the data of this first human exposure study with recombinant FSH (Org 32489, Organon International) suggest that it is a safe drug with pharmacokinetic properties comparable with those previously reported for natural FSH. Further clinical data will be required to confirm the safety and efficacy of recombinant FSH in infertile patients treated for induction of ovulation or for induction of controlled ovarian superovulation.

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