FERTILITY AND STERILITY®
VOL. 69, NO. 2, FEBRUARY 1998

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.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

Jean-Yves le Cotonnec, M.D., Hervé Claude Porchet, M.D.,* Vanya Beltrami, Ph.D., Alain Munafo, Ph.D.*

Ares Serono, Geneva, Switzerland

Objective: To assess the single-dose pharmacokinetics of a recombinant human LH preparation administered by the IV, IM, and SC route.

Design: Prospective, randomized cross-over study.

Setting: Phase I clinical research environment.

Patient(s): Twelve healthy pituitary down-regulated females.

Intervention(s): Subjects received single IV, IM, and SC doses of 10,000 IU of recombinant human LH, each separated by 1 week.

Main Outcome Measure(s): Pharmacokinetic parameters.

Result(s): After single IV administration, the pharmacokinetics were described by a two-compartment model, after IM or SC administration, by a one-compartment model with zero order absorption and a lag time. Using the immunoassay, after IV administration initial half-life was 1 hour and terminal half-life was 10 hours (half-life was prolonged after extravascular administration, suggesting rate-limiting absorption). Total serum clearance was 2.6 L/h, and steady-state volume of distribution was 14 L. Observed C_{max}, after IM and SC administration, was 43 IU/L with median t_{max} of 9 hours (IM) and 5 hours (SC). Bioavailability was 0.54 (IM) and 0.56 (SC). The pharmacokinetics of LH are comparable using an in vitro bioassay.

Conclusion(s): The terminal half-life of recombinant human LH is around 12 hours and is slightly prolonged after extravascular administration. The pharmacokinetics are similar after IM and SC injection, and one-half the administered dose is available systemically. (Fertil Steril® 1998;69:195–200. ©1998 by American Society for Reproductive Medicine.)

Key Words: Recombinant human LH, pharmacokinetics, immunoassay, in vitro bioassay, bioassay/immunoassay ratio

Received August 22, 1996; revised and accepted October 8, 1997.

Supported by a grant from Ares Serono International S.A., Geneva, Switzerland.

* Present address: Triskel Consulting, Arzier, Switzerland.

Reprint requests: Alain Munafo, Ph.D., Ares-Serono International S.A., 15 bis Chemin des Mines, Case Postale 54, 1211 Geneva 20, Switzerland (FAX: 41-22-739-33-30).

0015-0282/98/$19.00
PII S0015-0282(97)00502-5

In part I of this series (1), the pharmacokinetics of a new recombinant human LH preparation (LHadi, Laboratoires Serono, Aubonne, Switzerland) were described after IV administration and compared to those of urinary human LH (urinary hMG, Pergonal; Laboratoires Serono). Both compounds were found to have similar pharmacokinetics with a volume of distribution of 10 L, a total clearance of around 1.7 L/h, and thus identical elimination half-lives of around 10–12 hours.

In this second part we describe the pharmacokinetics of a high dose of recombinant human LH after single IV, IM, and SC administration; this latter regimen reflects one of the possible clinical uses of the drug (i.e., to mimic the preovulatory mid-cycle LH surge) (2).

The present study was designed to obtain the fundamental parameters necessary to characterize the pharmacokinetics of 10,000 IU of recombinant human LH after IV, IM, and SC administration.

MATERIALS AND METHODS

Subjects

Twelve pituitary down-regulated healthy female volunteers were enrolled in this study; these subjects were different from those studied in part I (1). Their mean age (± SD) was 24 ± 5 years, their mean (± SD) height was 165 ± 5 cm, and their mean (± SD) weight was 58 ± 6 kg. They met the following inclusion criteria: use of an oral contraceptive (OC) for contraceptive purposes only and not for regularization of the menstrual cycle; normal clinical and laboratory findings at the screening visit; normal electrocardiogram, blood pressure, and heart rate; body weight between 55 and 70 kg; regular menstrual cycles before taking the OC; smoking less than 10 cigarettes per day; negative drug abuse screening test. In addition, all subjects gave written informed consent.

The women first underwent pituitary desensitization by SC administration of a depot formulation containing 3.6 mg of the GnRH agonist (GnRH-a) goserelin (Zoladex, ZENECA, Macclesfield, United Kingdom). A second dose was given 28 days later to ensure the continuity of desensitization throughout the study. Subjects continued to take the OC until 7 days after the first goserelin dose. Immediately after completion of the study they were instructed to resume taking the OC, starting with a new pack.

The study was approved by the institutional ethics committee and was performed in accordance with the principles of the Helsinki Declaration.

Study Drug

Recombinant human LH (LHadi, Laboratoires Serono) was administered in a balanced, random-order, cross-over sequence, as a single dose of 10,000 IU in 1 mL of water for injection, on three occasions: IV, IM, and SC, each separated by 1 week of wash-out. The IV dose was given into the cubital vein of the opposite arm from which blood samples were withdrawn, the IM dose was given in the upper outer quadrant of the buttock, and the SC dose was administered into the anterior abdominal wall below the umbilicus.

Assay Methods

For measurement of LH, three analytical methods were used: 1) an in vivo bioassay (rat seminal vesicle weight gain assay as described by Van Hell et al. [3]) to determine the bioactivity of the injected material and its conformity with release specifications; 2) an in vitro immunoradiometric assay (LH MAIAclone assay, Biochem, Canada) to measure the immunoreactivity of the injected material and serum LH concentrations; and 3) an in vitro bioassay (MA10 Leydig cell bioassay) (4) to measure the LH activity of the injected material and serum LH concentrations.

The limit of sensitivity of the MA10 bioassay was 0.5 IU/L, and the intraassay and interassay coefficients of variation were 4% and 9%, respectively, in the laboratory performing the analysis for this study. The characteristics of the immunoassay have been described in part I (1).

Baseline Assessment

Immediately before the first dose of goserelin was administered and at least 2 weeks later, a 1-mL blood sample was collected to measure serum LH levels using immunoradiometric assay. The volunteers were considered eligible for the clinical phase of this study if their serum LH levels were <3.0 IU/L. Three days after confirmation of down-regulation, a 24-hour baseline serum LH profile was obtained. Blood samples of 4 mL were collected at 8:00 A.M., and at 1, 2, 4, 6, 9, 12, and 24 hours later. Blood was allowed to clot for 1 hour; it was then centrifuged, and serum was transferred into aliquots and stored frozen at −20°C to await analysis.

Clinical Phase

The first dose of recombinant human LH was given about 2 days after the baseline serum LH assessment. The schedule of testing and treatment was as follows. At 8:00 A.M., doses were administered to the subjects as described above. Samples of 4 mL of venous blood were drawn through an indwelling cannula at times that differed between the sessions: time 0 (before dose), 5 (IV only), 10 (IV only), and 15 minutes (IV only), and 0.5, 1, 2, 4, 5 (IM and SC only), 6, 9, 12, 24, 48, 72, 96, 120, and 144 hours after dosing. Blood samples were handled as described for baseline assessment.

No urine samples were collected since the IV study reported in part I showed that the renal clearance of recombinant human LH is very low, less than 4% of the systemic clearance.

Safety was assessed by direct questioning and by measurement of body temperature, heart rate, and blood pressure at 0 (predose), 2, 6, and 12 hours after dosing on the day of dosing and before blood collection on the other days.

Pharmacokinetic Analysis

As in the companion study (1), both noncompartmental and modelization approaches were used to analyze the data generated by the study. Exponential curve fitting was performed by nonlinear weighted least-square regression analysis (SIPHAR version 4.0, Simed, Créteil, France).

To take into account measurable concentrations of LH at time zero (before dose), because of endogenous (baseline) production of LH, the data were analyzed after correction for any predose concentration. Thus, the concentration at time zero was subtracted from the remaining data points within a given data set. This was done for both the model independent and the modeling analysis.

A monoexponential or biexponential model was fitted to the concentration time data, estimated with either the immunoradiometric assay or the in vitro bioassay, to obtain pharmacokinetic parameter estimates.

All data were fitted according to the following weighting function: $w_i = 1/C_i$, where C_i is the ith predicted serum

concentration of LH and w_i is the corresponding weight. This weighting function was chosen because the coefficient of variation of the measured concentrations decreases with increasing LH levels.

For the IV study data, a two-compartment model was chosen, with elimination from the central compartment. The models and equations used to describe the pharmacokinetics of recombinant human LH have been described previously (5).

For the single IM and SC dose, a one-compartment pharmacokinetic model was chosen, with zero-order absorption and a lag time, described by the following equation:

$$C = \frac{C_z}{\tau} \cdot \left(\frac{1 - e^{\theta \cdot \lambda_z}}{-\lambda_z}\right) \cdot e^{-\lambda_z(t - t_{lag})},$$

where t_{lag} is the lag time before absorption begins, τ is the estimated "zero order input duration" and $\theta = t - t_{lag}$, when $t < \tau + t_{lag}$ and $\theta = \tau$ when $t \geq \tau + t_{lag}$. This model is the result of convolution of the input function with a single monoexponential disposition function. To prevent the predicted concentration from falling below zero when $t < t_{lag}$, the statement "$C = 0$ for $t < t_{lag}$" was incorporated into the model.

The validity of the fittings was assessed by visual inspection of the fitted curve and observed data, by inspection of the residual plot versus time, and from the SE and confidence intervals of parameter estimates.

Additional pharmacokinetic parameters were derived from the parameter estimates obtained from the fitting procedure, according to standard methods (6). To compute pharmacokinetic parameters, the doses administered were measured with the same method used for measuring serum LH concentrations.

Statistical analysis was performed using the SPSS version 4 software package (SPSS Inc., Chicago, IL). The extravascular doses (IM and SC) were compared by multivariate analysis of variance (MANOVA). For these comparisons, it was assumed that the study was of a two-way crossover design. This retained balance in the study. The data were analyzed using the method of Hills and Armitage (7), testing for treatment effects, period effects, and treatment by period interactions. The parameters analyzed were C_{max}, area under the curve, and F, for both immunoassay and bioassay results.

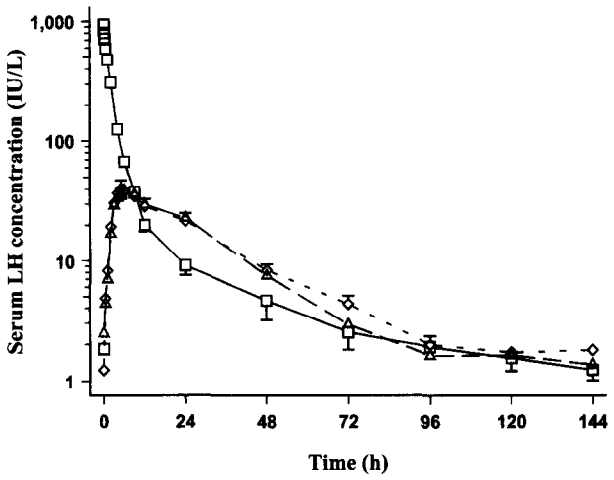
The parameter t_{max} for the extravascular data was compared by the nonparametric Wilcoxon's signed-rank test. Unless otherwise specified, data are presented as means ± SD.

RESULTS

The doses of recombinant human LH were well tolerated. No clinically significant changes in vital signs or laboratory test results were noted in any subject throughout the study. The main side effect was hot flushes attributable to the hypogonadism resulting from pituitary desensitization by the GnRH-a. Moderate headaches were reported by three volunteers. Effective gonadotropin suppression was achieved in all volunteers.

FIGURE 1

Log-linear plot of LH immunoassay concentrations versus time after single IV (solid line), IM (long dashed line), and SC (short dashed line) administration of 10,000 IU of recombinant human LH (mean ± 1 SEM, 12 subjects). For purposes of clarity, only a few error bars are presented.



Administered Dose

The vials of recombinant human LH had a nominal content of 10,000 IU as assessed by the in vivo bioassay, and the immunoradiometric assay and MA10 bioassay revealed LH contents of 5,600 IU and 10,280 IU, respectively. Because of the use of two different standards for the calibration of the immunoassay and of the bioassays, the results are not directly comparable since the LH units for the bioassay and the immunoassay are different (1).

Pharmacokinetic Results

The mean concentration–time profiles measured with the immunoassay for each treatment are shown in Figure 1. The profiles are essentially similar using the in vitro bioassay. The corresponding mean estimates of the pharmacokinetic parameters are shown in Table 1.

After IV administration, the LH concentration–time curves were adequately described by a two-compartment model and after IM and SC administration by a one-compartment model with zero-order absorption and a lagtime. In comparison with the LH concentrations reached after these doses, the observed concentration at time 0 (baseline) was almost negligible (1.4 ± 1.1 IU/L for immunoassay data, 1.9 ± 1.8 IU/L for bioassay data).

After single IV administration, the initial (distribution) half-life was around 1 hour and the terminal (elimination)

TABLE 1

Parameter estimates of LH pharmacokinetics (serum concentrations measured by the immunoassay and in vitro bioassay) after single IV, IM, and SC administration in 12 women.

Parameter (mean ± SD)	Immunoassay			In vitro bioassay		
	IV	IM	SC	IV	IM	SC
AUC (IU · h/L)	2,217 ± 500	1,118 ± 516	1,224 ± 480	4,472 ± 1,274	2,179 ± 672	2,466 ± 765
C_0 (IU/L)	863 ± 179	—	—	1,658 ± 222	—	—
C_{max} (IU/L)	—	45 ± 20	41 ± 24	—	74 ± 28	72 ± 39
t_{max} (h)*	—	9 (3–9)	5 (4–9)	—	6 (3–12)	5 (4–9)
Clearance (L/h)	2.6 ± 0.6	—	—	2.5 ± 0.6	—	—
t_{lag} (h)*	—	0.4 (0–0.9)	0.2 (0–0.5)	—	0.3 (0–1.8)	0.1 (0–0.7)
τ (h)	—	5.0 ± 1.4	4.4 ± 1.2	—	4.3 ± 2.1	4.1 ± 1.5
$t_{1/2\lambda_1}$ (h)	1.3 ± 0.3	—	—	1.0 ± 0.3	—	—
$t_{1/2}$ (h)	10 ± 5	16 ± 4	21 ± 5	19 ± 10	21 ± 6	24 ± 6
V_{ss} (L)	14 ± 5	—	—	31 ± 15	—	—
MRT (h)	6 ± 3	—	—	15 ± 11	—	—
Bioavailability (%)	—	54 ± 30	56 ± 23	—	51 ± 19	60 ± 20

Note. AUC = area under the concentration–time curve from time = 0 to infinity; C_{max} = maximal observed concentration over baseline; C_0 = estimated concentration at time 0; MRT = mean residence time; t_{max} = time of C_{max}; t_{lag} = lag time before absorption begins; τ = zero order input duration; $t_{1/2\lambda_1}$ = initial half-life; $t_{1/2}$ = terminal half-life; V_{ss} = volume of distribution at steady state.

* Median (range).

half-life was 10 and 19 hours as estimated from the immunoassay and bioassay data, respectively. The systemic serum clearance was approximately 2.5 L/h. The initial volume of distribution was found to be around 6 L for both analytical methods, whereas the volume of distribution at steady state, estimated from bioassay data, was double that from the immunoassay data. The mean residence time was also different for immunoassay (6 hours) and bioassay (15 hours), but a large degree of variability was observed.

The maximal increase of LH serum concentration over baseline was similar after single IM and SC administrations. It was reached at a median time of 5 hours with the SC route and at 9 hours with the IM route (not a statistically significant difference).

The lag time was rather short and the input duration was around 4 to 5 hours after both SC and IM administrations. Mean terminal half-life was 21 and 16 hours after SC and IM administration, respectively (24 and 21 hours from the bioassay data).

The mean systemic bioavailability was 0.55 with the IM and SC routes, as estimated from the immunoassay data. However, for bioassay data it was slightly, but significantly ($P = 0.04$), higher, for the SC route (0.6 versus 0.51).

There were no other statistically significant differences between the two extravascular doses or treatment by period interactions.

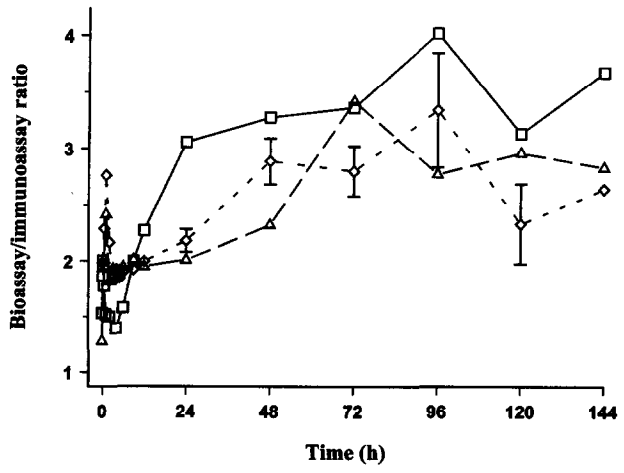
Bioassay to Immunoassay Ratio

The changes in the bioassay to immunoassay ratio after single IV, IM, and SC administrations are displayed in Figure 2. Before exogenous LH administration, the endogenous serum LH bioassay to immunoassay ratio averaged 1.5 ± 0.7. The recombinant human LH has a bioassay to immunoassay ratio of 1.9.

After IV, IM, and SC administrations of 10,000 IU of recombinant human LH, the serum LH bioassay to immunoassay ratio increased progressively to reach around 4.3 ± 2.2 by day 3 (median). Although this time of maximum bioassay to immunoassay ratio was rather variable, the ratio decreased thereafter but had not completely returned to baseline level by day 6.

FIGURE 2

Evolution over time of the in vitro bioassay/immunoassay ratio after single IV (solid line), IM (long dashed line), and SC (short dashed line) administrations of 10,000 IU of recombinant human LH (mean ± 1 SEM, 12 subjects). For purposes of clarity, only a few error bars are presented.



DISCUSSION

In a previous study (data on file, Ares Serono), LH serum profiles were monitored in healthy female volunteers during a spontaneous ovulatory cycle and after single administration of a GnRH-a to induce an LH surge. This study suggested that natural LH surges are associated with large interindividual variability and that the profile of the LH surge per se does not appear to be critical to induce effective ovulation. During spontaneous LH surges, immunoreactive peak concentrations (C_{max}) were around 50 IU/L, and their time of occurrence (t_{max}) was highly variable (6–40 hours), with an area under the concentration versus time curve around 1,000 IU · h/L. After administration of a single dose of GnRH-a, LH surges have a mean C_{max} around 130 IU/L, a t_{max} around 6 hours, and an area under the curve around 2,000 IU · h/L.

Two assay methods were used in this study to measure LH serum concentrations. The immunoassay can be considered a reliable analytical method and is well documented. However, the in vitro bioassay was performed in parallel to provide additional information about the LH bioactivity. Pharmacokinetic analysis focused on the results obtained with the MAIAclone immunoradiometric assay, and the MA10 Leydig cell in vitro bioassay results were used as supporting evidence.

The results indicate that recombinant human LH pharmacokinetics can be adequately described by a classic two-compartment model after IV bolus administration or by a one-compartment pharmacokinetic model with zero-order absorption and lag time after IM and SC administrations. Although LH profiles after these extravascular administrations could be fitted with more complex models, these would not be more informative than the simple one presented.

After IV administration, recombinant human LH undergoes a rapid phase of distribution with an initial half-life around 1 hour and is eliminated with a mean terminal half-life around 12 hours, as calculated from the immunoassay data.

Mean clearance, initial volume of distribution, and volume of distribution at steady state calculated from the immunoassay data are very similar to the results obtained in the first part of this series, where recombinant human LH was administered IV at doses ranging from 300–40,000 IU to a different group of 12 pituitary down-regulated healthy female volunteers (1).

After single IM and SC administration, the pharmacokinetics of LH were comparable, although the mean half-life estimate for the IM data was 20% shorter. Again the bioassay data yielded slightly longer half-life estimates than the immunoassay. These estimates were confirmed by model-independent, log-linear regression in the terminal portion of the curves (data not shown), and therefore, the observed differences are not attributable to model misspecification in the pharmacokinetic analysis.

The observation that, on immunoassay data, the terminal half-life after IM and especially SC administration is apparently longer than after IV administration, tends to indicate a rate-limiting absorption. Despite a larger variability in the half-life values, this was not observed with use of in vitro bioassay data. This suggests that there may be a differential absorption with respect to isoforms. Since immunoassay is less able to discriminate between isoforms than bioassay (as the isoforms may not have the same bioactivity), the above could be explained by the faster absorption of the more bioactive isoforms. In addition, baseline-corrected bioassay data allowed pharmacokinetic analysis to extend to later time points than the immunoassay, particularly after IV administration.

The ratio of bioassay concentration to immunoassay concentration generally increased over time (Fig. 2), also suggesting that the more bioactive form of LH remains in the body longer. This is further supported by the longer mean residence time estimated from the bioassay data. This appears to be attributable to a wider distribution volume, as reflected in the larger volume distribution at steady state, of the LH isoforms preferentially measured by the bioassay. A comparable observation from an earlier study of recombinant human follicle-stimulating hormone has been reported (8). An alternative explanation is a subtle nonlinearity between the two assay methods at low concentrations. It is not possible from this study to distinguish between the two explanations.

The maximum concentrations observed after IM and SC administrations were similar but occurred slightly earlier after SC administration. On the basis of the immunoassay data, IM and SC bioavailability are identical, although on the basis of the bioassay data the bioavailability is slightly in favor of SC administration. This observation may also be explained by differentiation with respect to isoforms.

However, overall the results obtained after IM and SC administrations are very similar and the two analytical methods give essentially comparable pharmacokinetic estimates. The apparent difference in C_{max} and area under the curve is only attributable to the fact that the immunoassay and the bioassay results are expressed in different units ("immunoactive" versus "bioactive" IUs). Dose normalized C_{max} and area under the curve are indeed identical for both analytical methods and both routes of administration.

In conclusion, the results of this study support the administration of recombinant human LH at relatively high doses. Based on immunoassay, the terminal half-life was found to be around 12 hours and was slightly prolonged after extravascular administration. When measured both by an immunoassay and an in vitro bioassay, the pharmacokinetics are very similar after single IM and SC injections and one-half of the administered dose is available systemically.

The results obtained using the bioassay data generally support those obtained using the immunoassay data. The

immunoassay can be considered a reliable (and physiologically meaningful) tool in the assessment of LH pharmacokinetics.

Acknowledgment: We thank Azhar Khan, Ph.D., and Steeve Toon, Ph.D. (Medeval Ltd., Manchester, England), as well as Malcolm Rowland, Ph.D. (University of Manchester, Manchester, England), for their valuable help.

References

1. Le Cotonnec J-Y, Porchet HC, Beltrami V, Munafo A. Clinical pharmacology of recombinant human luteinizing hormone. Part I. Pharmacokinetics after IV administration to healthy female volunteers and comparison with urinary-human LH. Fertil Steril 1998;69: 189–94.
2. Zelinski-Wooten M, Hutchison J, Chandrasekher Y, Wolf D, Stouffer R. Administration of human luteinizing hormone (hLH) to macaques after follicular development; further titration of LH surge requirements for ovulatory changes in primate follicles. J Clin Endocrinol Metab 1992; 75:502–7.
3. Van Hell H, Matthijsen R, Overbeek GA. Effects of menopausal gonadotrophin preparations in different bioassay methods. Acta Endocrinol (Copenh) 1964;47:409–18.
4. Van Damme M-P, Robertson DK, Diczfalusy E. An improved in vitro bioassay method for measuring luteinising hormone (LH) activity using mouse Leydig cell preparations. Acta Endocrinol (Copenh) 1974;77: 655–71.
5. Le Cotonnec J-Y, Porchet HC, Beltrami V, Kahn A, Toon S, Rowland M. Clinical pharmacology of recombinant human follicle-stimulating hormone (FSH). Part II. Single doses and steady-state pharmacokinetics. Fertil Steril 1994;61:679–86.
6. Gibaldi M, Perrier D. Pharmacokinetics. 2nd ed. New York: Marcel Dekker, 1982.
7. Hills M, Armitage P. The two period cross-over clinical trial. Br J Clin Pharmacol 1979;8:1349–77.
8. Le Cotonnec J-Y, Porchet HC, Beltrami V, Kahn A, Toon S, Rowland M. Clinical pharmacology of recombinant human follicle-stimulating hormone (FSH). Part I. Comparative pharmacokinetics with urinary human FSH. Fertil Steril 1994;61:669–78.