Comprehensive Pharmacokinetics of Urinary Human Follicle Stimulating Hormone in Healthy Female Volunteers

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Purpose. The study determined the pharmacokinetics of urinary human follicle stimulating hormone (u-hFSH) in 12 down-regulated healthy female volunteers. ***Methods.*** Following pituitary desensitization, baseline FSH serum levels were measured over a 24-hour period. Then each subject received, in random order, single doses of u-hFSH (Metrodin®), 75 IU, 150 IU and 300 IU iv, and 150 IU im on four occasions separated by washout periods of one week. Blood and urine samples were collected at preset times. FSH levels were measured by a immuno-radiometric assay and an *in vitro* rat granulosa cells aromatase bioassay. ***Results.*** All doses of u-hFSH were well tolerated. After an iv bolus, the pharmacokinetics of FSH were well described by a two-compartment open model. Immunoassay data showed that the total exposure to FSH was proportional to the administered dose. Mean total clearance of FSH was approximately 0.5 $L \cdot h^{-1}$ and renal clearance was 0.14 $L \cdot h^{-1}$. The volume of distribution at steady-state was around 8 liters. The distribution half-life was 2 h and the terminal half-life nearly one day. After im injection, almost two thirds of the administered dose was available systemically. The *in vitro* bioassay confirmed this pharmacokinetic analysis. ***Conclusions.*** The estimation of the elimination half-life of around one day indicates that the maximal effect of a given dose of u-hFSH administered daily cannot be observed until 3 to 4 days of repeated administration. This indicates that, on a pure pharmacokinetic basis, physicians should wait at least 4 days to assess the efficacy of a given dose of u-hFSH and that they should not modify dosage too frequently.

KEY WORDS: urinary human FSH; pharmacokinetics; immunoassay; in vitro bioassay; immunoassay:bioassay ratio.

INTRODUCTION

Complete or partial deficiency in FSH is a common cause of infertility in women and men. Administration of u-hFSH, either alone or in combination with LH, has been successful for treating these infertility problems since the early sixties. It is used over periods of 7-21 days to stimulate single follicular growth in hypothalamic-pituitary dysfunction (WHO group II, anovulatory women) or to stimulate multiple follicular growth in women receiving superovulation treatment for *in vitro* fertilization and other assisted reproductive technologies. The treatment of male infertility requires a therapy of at least six months. During the last twenty years, a number of reports estimating the half-life of urinary human follicle stimulating hormone (u-hFSH) have been published. In common with other hormones, a fast and a slow component of the half-life of u-hFSH, have been described. The initial rapid phase (distribution phase) represents disappearance of the hormone from plasma, whilst the terminal slow phase represents elimination from the body. The majority of reports agree that the distribution phase half-life is between 2 and 3h (1-7). However, there is no such consensus on the elimination half-life with reported elimination half-lives ranging from 7.3 h (6) to 70.4 h (3).

Better knowledge of the elimination half-life of u-hFSH would be important as a rational basis for establishing therapeutic schedules and to provide a reference for the development of recombinant hFSH. The present study was designed to establish in detail the pharmacokinetics of u-hFSH, measuring serum FSH levels simultaneously by a immunoradiometric assay and an *in vitro* bioassay.

MATERIALS AND METHODS

Subjects

The study was performed in twelve pituitary down-regulated, healthy female volunteers, who were taking an oral contraceptive before FSH therapy. Their mean age (±SD) was 22 ± 2 years, mean height 165 ± 5 cm and mean weight 62 ± 8 kg. All satisfied the following inclusion criteria: use of an oral contraceptive for contraceptive purposes only and not for regularization of the menstrual cycle; normal clinical and laboratory findings at the screening visit; normal ECG, blood pressure and heart rate; body weight between 55 and 70 kg; regular menstrual cycles prior to taking an oral contraceptive; smoking less than 10 cigarettes per day; negative drugs of abuse screen, and written informed consent.

The female volunteers were pituitary desensitized by subcutaneous administration of a depot formulation of Goserelin (Zoladex®, ICI Pharmaceuticals, UK). The study has been approved by the ethic committee of Manchester/ England and has been performed in accordance to the principles of the Helsinki Declaration.

Study Drug

Urinary human Follicle Stimulating Hormone (Metrodin®, Laboratoires SERONO, Aubonne / Switzerland) was administered in a balanced, random-order, cross-over sequence as a single dose on four occasions, each separated by one week of washout. The four doses were: 75 IU, 150 IU, 300 IU given iv, in the cubital vein of the opposite arm from which blood samples were withdrawn, and 150 IU given im in the upper outer quadrant of the buttock.

Assays Methods and Administered Dose

The FSH concentrations were determined by three methods (8, 9):

- An *in vivo* bioassay (Steelman-Pohley) to establish the bioactivity of the injected material and its conformity with

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release specifications. This assay ensures that each batch delivers a constant amount of pharmacodynamic activity independently of its isoform profile defined by differences in glycosylation.

- An *in vitro* bioassay (rat granulosa cell aromatase bioassay, GAB) to measure the FSH activity of the injected material and serum FSH concentrations (10). The limit of sensitivity was 2 $IU \cdot l^{-1}$ and the coefficient of variation of the measures (precision) was 5.5% at 5.5 $IU \cdot l^{-1}$ and 3.1% at 76 $IU \cdot l^{-1}$. The interassay coefficient of variation was 14%. Urinary FSH levels were not assayed with this assay as the *in vitro* bioassay gives inconsistent results in urine (K. Dahl, personal communication).
- An *in vitro* immuno-radiometric assay (FSH MAIAclone assay, Serono Diagnostics, Welwyn Garden city, UK) to measure the immunoreactivity of the injected material and serum and urine FSH concentrations. The MAIAclone assay incorporates two high-affinity antibodies into an immuno-radiometric assay (IRMA) system in order to attain higher sensitivity and specificity than with traditional RIA. In the laboratory which performed the analysis for this study, the limit of sensitivity of the MAIAclone was 0.7 $IU \cdot l^{-1}$ and the coefficient of variation of the measures (precision) was 8.5% at 6.1 $IU \cdot l^{-1}$ and 3.8% at 31 $IU \cdot l^{-1}$. The interassay coefficient of variation was <5.5% for serum and <13% for urine levels respectively.

All samples from one patient for one study period were analyzed in one batch. Both assays were recalibrated with the same standard and the potential effect of the GnRH agonist on the GAB was prevented by the addition of a GnRH antagonist (Antide) in the culture medium. Parallelism of the dose-responses curves for the IRMA and the GAB assays was established.

Administered Dose

The ampoules of u-hFSH used for this study had a measured content of 75 IU as assessed by the *in vivo* bioassay (Steelman-Pohley). The MAIAclone and *in vitro* bioassay (GAB), gave activities of 72.5 IU and 83 IU respectively. To compute pharmacokinetic parameters, the dose administered was determined by the same method for measuring serum or urine FSH concentrations.

Baseline Assessment

Just before the first Goserelin administration and ten days later, a 1 ml blood sample was collected to measure serum FSH levels by the immuno-radiometric assay. Three days after confirmation of down-regulation (FSH levels below 6.4 $IU \cdot L^{-1}$) a 24-h baseline serum FSH profile and FSH urinary excretion pattern were obtained. FSH samples were collected according to the same schedule as in the first 24 h of the drug treatment phase.

Clinical Phase

At 08.00h, the subjects were dosed as described above. Samples of 4 ml of venous blood were drawn through an indwelling cannula from the other arm at times 0 (pre-dose); 5 minutes (iv only); 15 minutes (iv only); 0.5; 1; 2; 4; 6; 9; 12; 24; 36; 48; 72; 96; 120 and 144 hours post-dose. Blood was allowed to clot for one hour at room temperature, centrifuged for 30 minutes at 2500 rpm, and the serum was divided into three aliquots of 500 µl and stored frozen at -20°C.

Urine was collected during the following post-dose intervals: 0-2h; 2-6h; 6-12h; 12-24h; 24-48h; 48-72h and 72-96h and the total volume of urine per interval was recorded. Two 1 ml specimens from each urine collection were stored frozen at -20°C to await further analysis.

Pharmacokinetic Analysis

Two complementary pharmacokinetic approaches were used to analyze the data generated by the study.

Exponential Model

The first approach involved the use of exponential models and a non-linear weighted regression analysis {PCNONLIN (version 3.0), SCI software, Lexington, USA}. A mono or biexponential model was fitted to the concentration time-data, estimated with either the immuno-radiometric assay or the *in vitro* bioassay, to obtain pharmacokinetic parameter estimates. In order to deal with the measurable concentrations of FSH at time zero, (pre-dose) due to endogenous (baseline) production of FSH, a so called baseline component was added to the model. This component assumes some monotonous variation in endogenous serum FSH concentration over each individual study period, but is independent from one period to another. The details of this approach has been previously reported by Le Cotonnec et al (11).

Additional pharmacokinetic parameters were derived from the parameter estimates obtained from the fitting procedure, according to standard methods (12). Renal clearance of FSH was calculated as the slope of the line of urinary FSH excretion rate plotted against the serum FSH concentration determined mid-way into the urine collection interval. The equations used to calculate the pharmacokinetic parameters were previously reported by Le Cotonnec et al (7).

Non-Compartmental Pharmacokinetics

For comparative purposes the pharmacokinetics of FSH were further analyzed using a non-compartmental (model-independent) approach, carried out using EXCEL 3.0 (Microsoft, Seattle, USA). In this instance the endogenous serum FSH concentration (baseline concentration) was subtracted from all post-dosing serum FSH concentrations thereby assuming a constant endogenous serum FSH over each study interval. Correcting for baseline FSH in this way sometimes resulted, at later time-points, in negative values for serum FSH concentrations or indeed concentration that increased with increasing time. Such data points were not included in subsequent non-compartmental analysis.

RESULTS

Effective suppression of serum FSH levels was achieved in all female volunteers. The doses of u-hFSH 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 due to the low estradiol (E_2) levels resulting from pituitary down-regulation by the GnRH agonist. Moderate headaches were

reported by two volunteers during a few hours after administration of 300 IU IV of u-hFSH

Immunoassay Data

The mean concentration-time profiles for each treatment are shown in figure 1 for the immunoassay data and the corresponding mean estimates of the pharmacokinetic parameters are shown in table 1. The immunoassay data, after iv administration were adequately described by a two compartment model and after im administration by a one-compartment model with first-order absorption. Both pharmacokinetic approaches produced comparable estimates of the pharmacokinetic parameters. The slope for endogenous FSH production was 0.004 ± 0.004 $IU \cdot h^{-1}$ i.e. 0.6 ± 0.6 IU during each one week sampling period and was not different from zero. The intercept at time 0 was 1.9 ± 0.9 $IU \cdot l^{-1}$. When a fixed horizontal baseline was subtracted, the mean value was 1.9 ± 1.0 $IU \cdot l^{-1}$. Serum FSH concentration returned to baseline in around 2, 3 and 4 days after the iv doses of 75, 150 and 300 IU respectively.

Based on the results with these three iv doses, the distribution half-life was 2 h and the true terminal half-life of FSH appeared to be roughly one day (19 ± 6h). The two pharmacokinetic approaches differed only slightly in their estimates of the terminal half-lives, the non-compartmental approach giving about a 20% longer half-life. A progressive increase in terminal half-lives was observed with both pharmacokinetic approaches, from 14 ± 9 to 19 ± 6 hours or from 17 ± 11 to 23 ± 5 hours respectively for the compartmental and the non-compartmental approaches (P = 0.3 ANOVA).

The increase in the drug exposure with doses, as measured by the area under the serum FSH concentration-time curve, was slightly larger than the increase in doses: 150 ± 30 $IU \cdot h \cdot l^{-1}$ and 160 ± 40 $IU \cdot h \cdot l^{-1}$ for the dose of 75 IU, but 740 ± 130 $IU \cdot h \cdot l^{-1}$ and 820 ± 160 $IU \cdot h \cdot l^{-1}$ for a 300 IU dose, for the compartmental and the non-compartmental approaches respectively (P = 0.06 ANOVA).

The estimated total clearance of 0.5 $l \cdot h^{-1}$ was similar for all three doses. The renal clearance, around 0.15 $l \cdot h^{-1}$, amounted to approximately 30% of total clearance, indicating that one third of the u-hFSH dose is excreted in urine. This proportion appeared to be independent of the dose administered. Thus moderate renal impairment will not request dosage adjustment for hFSH. Renal clearance of FSH, falling clearly below the glomerular filtration rate, indicates tubular reabsorption.

The absolute bioavailability of u-hFSH when injected im indicates that approximately two thirds of the administered dose is absorbed.

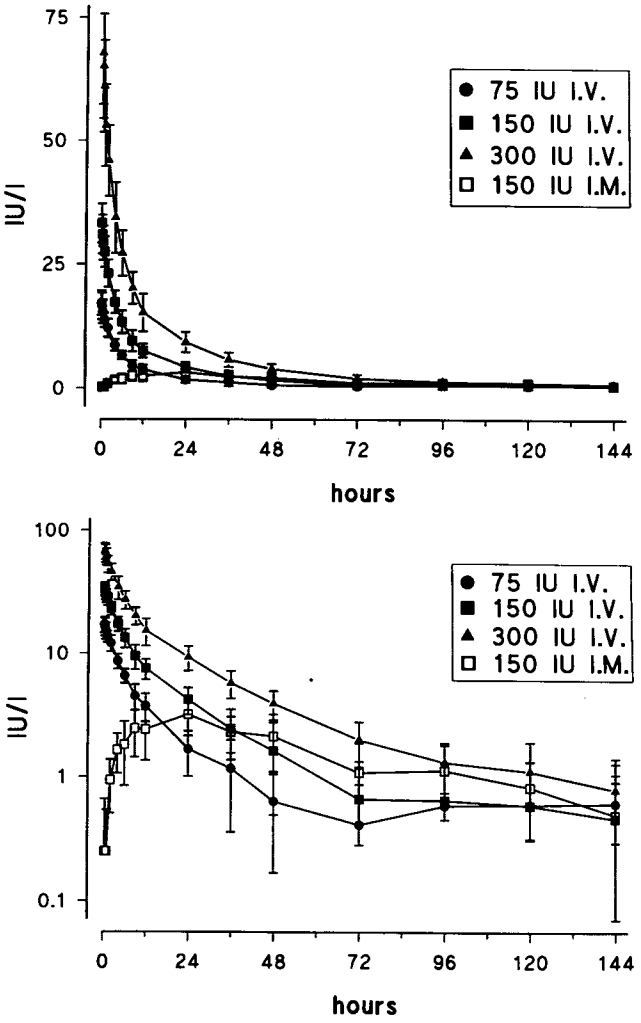

Fig. 1. Concentration-time profiles of FSH for immunoassay data. (mean of 12 subjects ± SD; Linear and Log-Linear plots)

In Vitro Bioassay Data

The mean concentration-time profiles for each treatment for the *in vitro* bioassay data (data not shown) were similar to the one observed for immunoassay data and the corresponding mean estimates of the pharmacokinetic parameters are shown in table 2. The same models as for immunoassay data were used to describe the *in vitro* bioassay data. However as variability in this data set was much greater (around 40% versus 20%), the estimates of the different parameters should be considered with more caution. Because of this wide variability it was not reasonably possible to fit a model to the im data. Thus we only report the parameters estimated with the non-compartmental approach for the im route.

The estimated average systemic bioavailability of FSH after im administration based on the bioassay data was approximately 30% (±20%). However, on account of the heterogeneity of this data set and as bioavailability could be calculated properly only in five volunteers out of 12, this estimate cannot be considered reliable.

Bioassay Versus Immunoassay Data

As previously mentioned, we based our pharmacokinetic analysis mainly on the immunoassay data, but it is worth noting that both assays give the same trends within the data. Substantial differences in parameter estimates with the two assays clearly exist with the bioassay giving a much larger $AUC_{0-\infty}$ and hence smaller clearance, and a longer terminal half-life. In this respect it is interesting to note that the plot (figure 2) of the mean FSH concentrations estimated using the two assays shows signs of non-linearity. This is particularly true with the 300 IU dose. This suggests that the bio-

Table 1. Parameter Estimates According to Compartmental and Non-compartmental Pharmacokinetic Analysis of the Immunoassay Data[a]

	Parameter							
	Compartmental				Non-compartmental			
Labeled dose (IU)	75	150	300	150	75	150	300	150
Immunoassay dose (IU)	72.5 iv	145 iv	290 iv	145 im	72.5 iv	145 iv	290 iv	145 im
$AUC_{0-\infty}$ (IU · h · l^{-1})	150	360	740	210	160	370	820	270
±SD	±30	±70	±130	±100	±40	±90	±160	±100
C_{max} (IU/l)	17	33	68	3	17	33	68	3
±SD	±3	±3	±10	±1	±3	±4	±10	±1
T_{max} (h)				18				23
±SD				±6				±10
CL (l/h)	0.5	0.4	0.4		0.5	0.4	0.4	
±SD	±0.1	±0.1	±0.1		±0.1	±0.1	±0.1	
CL_{renal} (l/h)	0.17	0.14	0.14					
±SD	±0.05	±0.03	±0.04					
$t_{1/2}$ distribution (h)	2	2	2					
± SD	±1	±1	±1					
$t_{1/2}$ terminal (h)	14	17	19	29	17	18	23	60
±SD	±9	±5	±6	±14	±11	±7	±5	±28
V_{ss} (l)	7	8	9		7	8	9	
±SD	±2	±1	±2		±3	±2	±2	
MRT (h)	15	19	22		17	20	26	
±SD	±6	±5	±5		±8	±8	±5	
F (%)				58				74
±SD				±25				±26

[a] $AUC_{0-\infty}$ area under the concentration-time curve from time 0 to infinity; C_{max} maximal concentration; T_{max} time of maximal concentration; CL total clearance; CL_{renal} renal clearance; $t_{1/2\ distribution}$ distribution half-life; $t_{1/2\ terminal}$ terminal half-life; V_{ss} volume of distribution at steady state; MRT mean residents time; F systemic bioavailability.

active FSH concentrations decline more slowly than the immunoreactive FSH concentrations. In other words that there is a progressive time-dependent increase in the FSH bioassay/immunoassay (B/I) ratio. The result is a hook-shaped curve with deviation from linearity occurring at low concentrations (which correspond to late sampling times). Similar findings were reported by Jia (13). These time-dependent changes in the immuno/bioassay relationship reflect changes in the biological characteristics of FSH.

This observation partly explains the differences between immunoassay and bioassay estimates of the terminal half-life, $AUC_{0-\infty}$ and clearance, which all depend to a greater extent on the low late concentrations. No such differences were observed for the initial (distribution) half-life which is dependent only on the early high concentrations.

This time-dependent changes in the FSH B/I ratio is more apparent in figure 3 which displays the time course of the B/I ratio. Before administration the endogenous serum FSH B/I ratio was around 4 ± 0.7 (mean ± SE). As exogenous u-hFSH has a B/I ratio of 1.15, the serum FSH B/I ratio drops steady immediately after the iv bolus. This drop is dose-related. Five minutes after 150 and 300 IU of u-hFSH, the new B/I ratios are 3.4 ± 0.3 and 2.4 ± 0.2 respectively, corresponding to the blend of exogenous u-hFSH and endogenous FSH. The serum FSH B/I ratio remains unchanged for about 2 h, then progressively increases with time to 7 ± 1 around day 4. Thereafter, the ratios decrease, returning to normal by day 7 when presumably all exogenous FSH was eliminated.

This suggests that after an initial dilution phase, progressive metabolic activation or selection of u-hFSH occurs. Findings after the im dose were similar but changes were slower and the coefficient of variation were far greater because of the low levels of serum FSH (data not shown).

DISCUSSION

Preliminary Considerations

Pharmacokinetic analysis of endogenous substances is always difficult because of their unknown fluctuations after exogenous administration though these can be minimized by blocking endogenous production as far as possible. In this study, we used chronic administration of a GnRH agonist to suppress endogenous FSH production and in fact a stable baseline was obtained in each volunteer for the total duration of the study. Between doses, each volunteer returned to its baseline level of FSH. In addition, a randomized cross-over design was used to balance any possible carry-over effect.

Two assay methods were used to measure FSH serum concentrations. Immunoassay can be taken as the reference analytical method because it is well documented, has been widely used and allow measurements of FSH concentrations in urine. However we employed the *in vitro* bioassay as a complementary method because, although used less often, it may provide auxiliary information about the pharmacokinetic behavior of FSH bioactivity.

Pharmacokinetics of u-hFSH in man

The results indicate that u-hFSH pharmacokinetics can be adequately described by a classical two compartment model after iv bolus administration or by a one-compartment pharmacokinetic model with first-order absorption following im administration.

Table 2. Parameter Estimates According to Compartmental and Non-compartmental Pharmacokinetic Analysis of the in Vitro Bioassay Data[a]

	Parameter							
	Compartmental				Noncompartmental			
Labeled dose (IU)	75	150	300	150	75	150	300	150
In vitro bioassay dose (IU)	83 iv	166 iv	332 iv	166 im	83 iv	166 iv	332 iv	166 im
AUC 0-∞ (IU · h · l-1)	500	1630	3090		530	2230	3990	570
±SD	±300	±600	±960		±350	±730	±1190	±380
Cmax (IU/l)	42	107	158		41	113	158	5
±SD	±13	±39	±33		±13	±41	±30	±4
Tmax (h)								27
±SD								±44
CL (l/h)	0.25	0.12	0.12		0.4	0.08	0.09	
±SD	±0.2	±0.05	±0.05		±0.5	±0.02	±0.03	
$t_{1/2}$ distribution (h)	2	2	2					
±SD	±1	±1	±2					
$t_{1/2}$ terminal (h)	28	29	32		27	42	44	83
±SD	±20	±7	±15		±20	±15	±17	±59
Vss (l)	6	4	5		5	4	5	
±SD	±3	±1	±2		±2	±1	±2	
MRT (h)	32	36	42		32	54	60	
±SD	±22	±10	±18		±23	±21	±24	
F (%)								28
±SD								±19

[a] $AUC_{0-\infty}$ area under the concentration-time curve from time 0 to infinity; C_{max} maximal concentration; T_{max} time of maximal concentration; CL total clearance; $t_{1/2\ distribution}$ distribution half-life; $t_{1/2\ terminal}$ terminal half-life; V_{SS} volume of distribution at steady state; MRT mean residence time; F systemic bioavailability.

Terminal Half-Life

Following iv administration u-hFSH undergoes a phase of distribution with an initial half-life around 2 h and then is eliminated with a terminal half-life around one day. It should be pointed out that iv administration is the only route were the estimation of the terminal half-life is not contaminated by the absorption phase thus providing a reliable evaluation.

The differences observed between the two pharmacokinetic approaches in estimating the terminal half-lives are a direct effect of the baseline modeling which slightly increases with the compartmental model but is constant with the non-compartmental approach. As neither of these pharmacokinetic approaches can be shown to be more correct from a physiological point of view, each absolute estimate of the terminal half-life of FSH must be considered with some caution. But as this bias is present in the analysis of each treatment, it does not fundamentally preclude comparison of treatments. The apparent increase in terminal half-life with dose of u-hFSH may arise because FSH concentrations return to the baseline level sooner after the lower dose (75 IU), so that the interval over which one can estimate the terminal half-life is shorter after the lower doses than after the larger

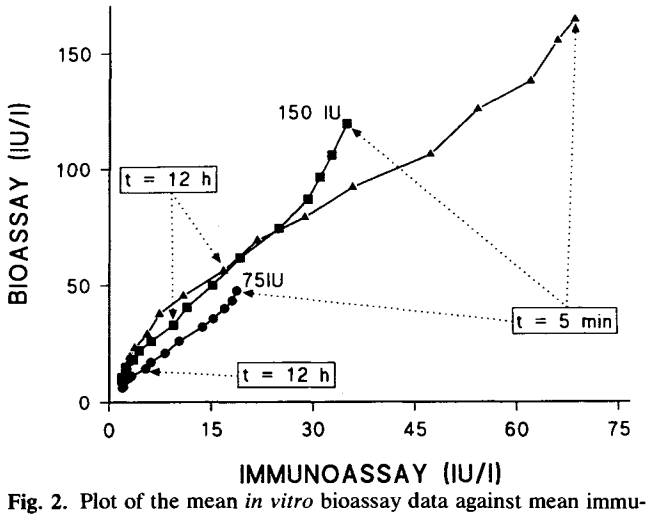

Fig. 2. Plot of the mean *in vitro* bioassay data against mean immunoassay data according to each intravenous dose of u-hFSH.

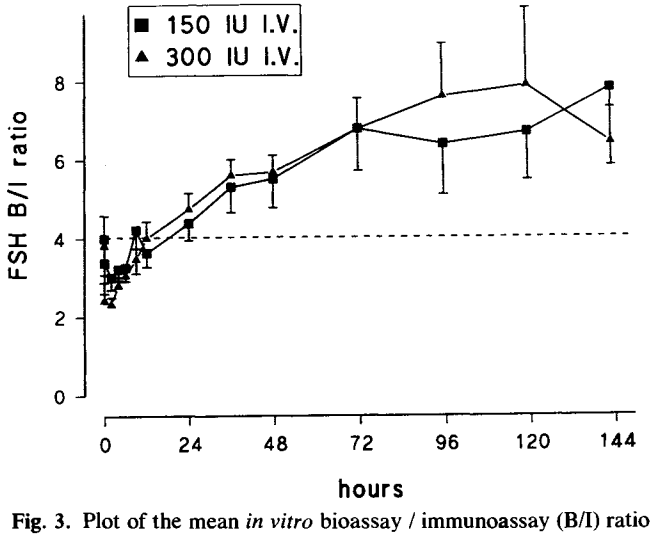

Fig. 3. Plot of the mean *in vitro* bioassay / immunoassay (B/I) ratio against time after intravenous administration of u-hFSH.

dose. This leads to underestimation of the terminal half-life at low doses.

The slight, non-proportional increase in AUC should not be interpreted as evidence of the non-linearity of FSH pharmacokinetics. If we consider figure 4, which depict the individual $AUC_{0-\infty}$ computed according to the non-compartmental approach as a function of the dose, total drug exposure is roughly proportional to doses. In the light of the wide dispersion of the $AUC_{0-\infty}$ values (inter-individual variability), especially with the highest dose, it is almost impossible here to draw any definite conclusion. If non-linearity does exist (around 20%), though, it should have little clinical relevance compared to inter-individual variability (around 40%).

After single im administration the estimation of the apparent terminal half-life is somehow longer (29 ± 14 h) than after iv administration. The estimates after im administration must be interpreted with caution as the model fitted to the data may not be absolutely correct. It is possible that terminal half-life was overestimated if some u-hFSH is still absorbed later in time. This is also indicated by the great interindividual variability (50%) observed on this parameter. However, a "flip flop" effect can be excluded as the estimated absorption half-life is much too short (5 ± 5 h) to be confounded with the terminal half-life. Additionally, with this mode of administration, the peak concentrations (3 and 5 IU/l) after subtraction of the baseline (2 and 6 IU/l for the immunoassay and the GAB respectively) are relatively low, and thus the baseline component plays a larger role than after iv administration. This is well illustrated by the larger interindividual differences between the estimates with the two pharmacokinetic approaches. Clearly, no such difference is observed after iv administration where concentrations are much higher.

Previous studies of hFSH found elimination half-life ranging from 7.3 to 70.4 h (1-7) with wide variability. On the contrary we obtained a more precise estimate with an acceptable variability (around 30 % iv and 50 % im). This is due unequivocally to the experimental setting that we utilized where a relatively large number of volunteers were observed over an extended period of time in a stable condition where endogenous FSH production had been arrested following pituitary desensitization. Interestingly this situation is similar to the clinical condition encountered in most IVF cycles offering thus clinical relevance to our conclusions.

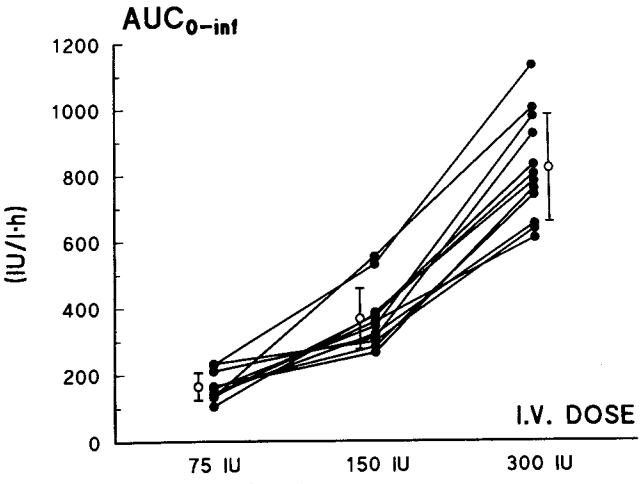

Fig. 4. Individual plots of $AUC_{0-\infty}$, computed according to the non-compartmental pharmacokinetic analysis, of the immunoassay data as a function of the intravenous dose of FSH. Open symbols represent mean value for each dose ±SD.

Bioavailability

The absolute bioavailability of u-hFSH after im administration is around 2/3 when FSH serum levels are measured with a immuno-radiometric assay.

Clearance

After iv administration in both parts of this study, the estimated total clearance was 0.5 ± 0.1 $L \cdot h^{-1}$ while the renal clearance was estimated at 0.14 ± 0.04 $L \cdot h^{-1}$. Around 30% of the administered u-hFSH dose was excreted into the urine, an observation in agreement with results reported by Amin (14). The renal clearance of hFSH is less than the glomerular filtration rate (GFR). This may indicate that hFSH is reabsorbed following filtration, or that high molecular weight glycosylated hFSH (MW around 31 kD) are too large to be excreted freely. Renal metabolism of the molecule could also be an explanation for the observed low renal clearance.

Volumes of Distribution

The initial volume of distribution (V_c) was found to be around 4 L which corresponds well to serum volume. The volume of distribution at steady state (V_{ss}) was around 8 L which approaches the volume of extracellular water.

Bioassay Versus Immunoassay Data

As recognition of the FSH molecules by the immunoassay is much less influenced by FSH glycosylation than the bioassay, and as the injected u-hFSH is a mixture of several isoforms defined by differences in glycosylation, at least two hypothesis can be formulated:

(i) The removal rate of the isoforms with high potency is slower than for the isoforms with low biopotency, leading to a progressive increase in the B/I ratio. This is probably not the case given that desialated FSH which are basic isoforms, have high potency *in vitro* but very short half-life *in vivo*, while sialated acidic isoforms, have a lower potency *in vitro* but are protected from hepatic metabolisation and thus have prolonged half-life *in vivo*.

(ii) Some progressive metabolic changes occur in the circulating FSH population, leading to an increase in potency that is only detectable with the *in vitro* bioassay. This would also partly explain the non-linearity observed with the bioassay for clearance and terminal half-life. This second hypothesis fits better with the current knowledge on the role of carbohydrate moieties, and particularly the sialic acid content on FSH activity and clearance.

Neuramidase activity, ubiquitous throughout the body, progressively desialates the FSH molecule after administration (15). This results in more basic isoforms with increased receptor binding affinity and higher potency as measured by the *in vitro* bioassay but not in the immunoassay (16-18).

This might explain the increase in serum FSH B/I ratio observed by Jia (13) during the luteal phase of the menstrual cycle compared to the follicular phase. The endogenous FSH peak which occurs around ovulation is metabolized progressively during the next 7 to 10 days, leading to a higher B/I ratio during the luteal phase.

However, as larger interindividual variations in pharmacokinetic parameters are obtained with the bioassay than with the immunoassay some caution should be applied before drawing too definite conclusions on the differences in kinetics using the two assay methods. Isofocusing electrophoresis of the serum samples might be a basis for reaching the right explanation. Overall, the results of the *in vitro* bioassays do largely confirm the pharmacokinetic analysis of the immunoassay data.

CONCLUSIONS

The estimation of the elimination half-life of around one day indicates that the maximal effect of a given dose of u-hFSH administered daily cannot be observed until 3 to 4 days of repeated administration. This indicates that, on a pure pharmacokinetic basis, physicians should wait at least 4 days to assess the efficacy of a given dose of u-hFSH and that they should not modify dosage too frequently.

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