Studies on pharmacokinetics of recombinant human follicle stimulating hormone

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Abstract
Follicle stimulating hormone (FSH) is a heterodimeric glycoprotein hormones secreted by anterior pituitary gland and is composed of two non-covalently linked protein subunits, the α-subunit which is common to other glycoprotein hormones, and the a hormone-specific β-subunit. FSH preparation plays an essential role in the treatment of human infertility. Recombinant human follicle stimulating hormone (r-hFSH) is now produced by recombinant DNA technology and has been introduced to the market. Pharmacokinetics studies of single and repeated dose were conducted in rat and monkey. Since u-hFSH and r-hFSH have similar pharmacokinetic profiles in vivo in both animals and humans. Toxicokinetic and clinical studies however confirmed that animals had been exposed to high levels of r-hFSH despite antibody formation. In this review, all relevant pharmacokinetic and pharmacodynamic information of FSH and r-hFSH provides a scientific basis and is valuable for reasonable application of the product.

Key words
Follicle stimulating hormone; recombinant human follicle stimulating hormone; pharmacodynamics; pharmacokinetics; toxicokinetics

Introduction
Follicle stimulating hormone (FSH) is a heterodimeric glycoprotein hormones secreted by anterior pituitary gland and is composed of two non-covalently linked protein subunits, the α-subunit (92 amino acids) which is common to other glycoprotein hormones, and the a hormone-specific β-subunit (111 amino acids)¹². FSH preparation plays an essential role in the treatment of human infertility. Recombinant human follicle stimulating hormone (r-hFSH) is now produced by recombinant DNA technology and has been introduced to the market. It is widely used in
treatment of anovulatory infertile patients and in stimulating multiple follicle development and maturation in patients undergoing assisted reproductive treatment (ART)\(^3,4\). r-hFSH was produced by Chinese hamster ovary cell lines transfected with the genes encoding for the two subunits of FSH\(^5,6,7\). r-hFSH has been shown to be similar to urinary-human follicle stimulating hormone (u-hFSH) in amino acid sequence, glycosylation sites, receptor binding activity, and in-vitro biological activity\(^7,8,9\), and it is likely that urinary-derived FSH preparation will be replaced by recombinant products for stimulating ovarian follicular development. The use of recombinant FSH offers advantages over urine-derived preparations, because it has been to bring to clinic more consistent, better defined, safer, more user-friendly, mono-therapeutic preparations. Compared with the u-rFSH, r-hFSH has the following characteristics: it is independent from urine production process and avoids any shortage of materials for clinical use due to lack raw materials; r-hFSH preparation has a high purity and specific activity (>99% pure, specific activity more than 10,000 IU/mg protein), which allows for safe administration through the s.c. route, with a good local tolerance to injection and a low immunogenicity\(^10,11\), good batch to batch consistency can be achieved because the manufacturing process of a recombinant DNA technology product can be better controlled; r-hFSH preparation is free of LH activity, providing a mono-therapeutic agent for clinical use. The production of r-hFSH by recombinant DNA technology, has the following characteristics: it is a urine-independent production process; good batch-to-batch consistency is ensured; effective purification takes place; absence of LH activity; and the final product is suitable for subcutaneous injection.

FSH papers are increasing, up to more than 16.8 thousand articles, the r-hFSH have more than 600 articles, of which the country 300 papers on pharmacokinetics study. Recent clinical research is also very active, many authors published a very valuable clinical research findings\(^12-14\) his reviews all relevant pharmacokinetic and pharmacodynamic data of FSH which were mainly obtained in volunteers.

**Preclinical pharmacokinetics studies**

Pharmacokinetics studies of single and repeated dose were conducted in rat and monkey in preclinical studies. Since u-hFSH and r-hFSH have similar pharmacokinetic profiles *in vivo* in animals. With pituitary FSH and r-hFSH, the most sialylated isoforms have the longer half-lives and are the more active forms *in vivo*. Toxicokinetic studies however confirmed that animals had been exposed to high levels of r-hFSH despite antibody formation. There was general similarity of toxicological findings in the shorter and longer-term studies, which represent the pharmacological actions of high doses of FSH, demonstrating evidence of the continuous exposure to r-hFSH despite antibody formation. There was no pathological evidence suggestive of the formation of immune complexes.

Klein studied comparatively the pharmacokinetics of a long-acting FSH analog containing the hCG-β carboxyterminal peptide, recombinant hFSH–CTP (r-hFSH–CTP) with r-hFSH and describe the pharmacodynamics of r-hFSH–CTP after SC injection in female rhesus monkeys. r-hFSH and r-hFSH–CTP were administered via a single SC or IV dose to rhesus monkeys, and serial phlebotomy was performed. An additional monkeys were pretreated with SC ganirelix and received SC r-hFSH–CTP after confirmation of pituitary suppression. Plasma disappearance rate of recombinant hFSH and r-hFSH–CTP and serum estradiol levels were analyzed. The elimination half-life of r-hFSH–CTP was twofold and fourfold longer than that of r-hFSH after SC and IV dosing, respectively. The absorption half-life was approximately threefold longer for r-hFSH–CTP than for r-hFSH after SC administration. R-hFSH–CTP stimulates estradiol secretion for 5–7 days after an isolated SC dose. Addition of the hCG-β carboxyterminal peptide to hFSH-β results in an FSH analog with longer absorption and
elimination half-lives compared with native hormone. This analog is capable of prolonged ovarian stimulation in rhesus monkeys after an isolated SC injection.\textsuperscript{[15]}

**Clinical pharmacokinetics studies**

Mannaerts et al. presented a historical overview on pharmacokinetic model of rFSH.\textsuperscript{[16]} The first human exposure studies of rFSH go back to early 1991, when single and multiple-rising doses were administered to gonadotrophin-deficient but otherwise healthy female and male volunteers.\textsuperscript{[17]} These phase I studies were performed in four specialist reproductive endocrinology and infertility units. Apart from safety and pharmacokinetic data on rFSH, these studies provided for the first time information on the pharmacodynamics of FSH in the absence of luteinizing hormone (LH). Furthermore, it has been shown that the bioavailability of FSH after the administration of exogenous FSH is influenced by many factors, including gender, body weight and route of administration.

In late 1991, a phase II efficacy study was initiated in women undergoing in-vitro fertilization and embryo transfer to evaluate whether rFSH therapy could be combined with various gonadotrophin-releasing hormone (GnRH) agonist treatment regimens, inducing different degrees of pituitary suppression. The efficacy data indicated that rFSH stimulates multiple follicular development with corresponding rises in serum inhibin (LH-independent) and oestradiol (LH-dependent) concentrations. The rise in oestradiol concentration indicates that in women with normal menstrual cycles the amount of remaining endogenous LH after profound pituitary suppression (1–2 IU·L\textsuperscript{-1}) is still sufficient to support rFSH-induced oestrogen biosynthesis.\textsuperscript{[18]} The outcome of this pilot efficacy study justified the further development of rFSH by means of several randomized, group-comparative, phase III studies.

**Single-dose pharmacokinetics**

In gonadotrophin-deficient volunteers, who received a single i.m. injection of 300 IU rFSH in the buttock,\textsuperscript{[19]} the rate of FSH absorption was lower in women than in men, with lower peak FSH concentrations ($C_{\text{max}}$), a longer time interval to peak serum FSH concentration ($t_{\text{max}}$) and a smaller area under the serum concentration versus time curve (AUC), with the AUC tending to be smaller in women compared with men. These increases in serum FSH measured after the single administration of rFSH or urinary FSH are presented in Fig 1. The calculated elimination half-life ($t_{1/2}$) of rFSH was 30–40 h and not significantly different between the sexes or between treatments.

![Fig 1. Mean curves of serum immunoreactive FSH after a single dose of 300 IU recombinant and urinary FSH in gonadotrophin-deficient women (upper panel) and men (lower panel).][19]

**Multiple-dose pharmacokinetics**

Two multiple-dose studies of rFSH were performed, i.e. one study in gonadotrophin-deficient male and female volunteers and the other study in female volunteers pituitary suppressed by Lyndiol. In the first study, the safety of rFSH was the main objective, but in addition information on the pharmacokinetics and pharmacodynamics of rFSH was gathered. The second study focused mainly on the pharmacokinetic and pharmacodynamic
properties of r-FSH in comparison with those of urinary FSH. During the multiple-rising dose study of rFSH in gonadotrophin-deficient subjects, the dose was increased at weekly intervals: the first 7 days, 75 IU·d⁻¹; the subsequent 7 days, 150 IU·d⁻¹; and the last 7 days, 225 IU·d⁻¹. Serum FSH concentrations increased in a dose-dependent manner, and steady state levels were reached after 3–5 days of treatment.[20] As a typical example, the individual serum immunoreactive FSH concentration measured in three gonadotrophin-deficient men are presented in Fig 2. An analysis of individual steady state levels and the body weight of all male volunteers indicated a negative correlation and supports the previously revealed negative correlation between Cmax and body weight after single-dose administration. Accordingly, one subject weighing only 42 kg had relatively high increments of serum FSH.

![Graph of FSH (IU) vs. time (weeks) for three subjects with body weights of 42, 56, and 71 kg.](image)

**Fig 2.** Individual graphs of serum immunoreactive FSH measured in three gonadotrophin-deficient men treated with single daily i.m. doses of recombinant FSH for 3 weeks. Doses were increased at weekly intervals from 75 to 225 IU/day.[19]

One phase I clinical study compared r-hFSH and natural u-FSH. It was performed in a group of 12 healthy women after pituitary desensitisation with GnRH agonists and shows that a single 150 IU daily r-hFSH dose for 7 days is effective in inducing follicular growth. The inter-individual variation observed was considered by the rapporteur related to variable ovarian sensitivity, rather than differences in FSH pharmacokinetics.

Human gonadotrophin preparations have been used in the treatment of infertility for almost four decades. The earliest FSH preparations were derived from urine from postmenopausal women and contained approximately equal amounts of FSH and luteinizing hormone (LH) activities. However, with the recognition that FSH is the principal regulator of follicular growth and maturation, these have been largely superseded by highly purified urinary FSH preparations and, more recently, r-hFSH. In general, these have shown that fewer FSH ampoules are required to achieve ovarian stimulation with r-hFSH, while the number of oocytes retrieved and embryos produced are higher than with urinary FSH. Additionally, the results of a recent meta-analysis have also shown that the clinical pregnancy rate per cycle started is significantly higher with r-hFSH, compared with urinary FSH. Furthermore, in poor responder patients, higher implantation rates were seen in patients treated with r-hFSH than in those treated with urinary FSH. The finding that FSH preparations produce effective ovarian stimulation compared to human menopausal gonadotrophins in women undergoing ART raises the question of whether LH is required for ovarian stimulation. It is suggested that r-hFSH is a consistently pure and high quality gonadotrophin preparation and contributes to increasing the successful outcome of an ART cycle.[21]

Hugues et al reported an improvement in consistency of response to ovarian stimulation with recombinant human follicle stimulating hormone resulting from a new method. They designed and conducted to assess the clinical relevance of this new method for quantifying therapeutic preparation of FSH. Four bulk lots of r-hFSH were used to prepare batches filled by IU (FbIU) and four batches filled by mass (FbM). These eight batches were compared in a double-blind, randomized study in patients undergoing assisted reproductive technology. One hundred and thirty-one patients were enrolled in this study and met protocol criteria. The starting dose of r-hFSH was either 150 IU or 11 μg·d⁻¹. Both preparations induced multiple follicular development and all patients underwent oocyte retrieval. The number of follicles ≥11mm.
was 14.85 and 14.91, serum oestradiol concentration on day of human chorionic gonadotrophin (HCG) administration was 6524 and 6350 pmol·L⁻¹, number of oocytes retrieved was 10.76 and 11.28, number of two-pronuclear oocytes was 5.2 and 5.00, number of viable embryos was 4.15 and 3.72, and clinical pregnancy rate was 30.3 and 26.2% respectively in the FbM and FbIU groups. Overall, the patients' response consistency was found to be superior with FbM, and in particular for clinical pregnancy rates. This new method for quantifying r-hFSH delivers an improved consistency in clinical outcome.[22]

Population pharmacokinetics

The information about many of the model parameters is sparse and therefore the population approach has an advantage over traditional pharmacokinetic methods since it pools the available information across many subjects. To characterize of the pharmacokinetics of r-hFSH and u-hFSH was study by using population pharmacokinetic analysis and deconvolution techniques. Sparse data were available from 62 female patients who received u-hFSH intramuscularly (i.m.) and 60 female patients who received r-hFSH subcutaneously (s.c.) as part of an in vitro fertilisation and embryo transfer (IVF-ET) procedure.[23] The dose of u-hFSH and r-hFSH was 225 International Units (IU) FSH/day for the first 5 days of treatment. The dose of u-hFSH/r-hFSH on subsequent days depended upon the ovarian response. Intensively sampled data were also available from 12 female volunteers who received r-hFSH, 150 IU, on three occasions: intravenously (i.v.), i.m. and s.c., each separated by 1 week of wash-out. The volunteers then received multiple r-hFSH doses by the s.c. route: 150 IU once daily for 7 days. Intensively sampled data were available from a further 12 female volunteers who received u-hFSH, 150 IU, given by the i.v. and i.m. routes.

Analysis of the intensively sampled r-hFSH and u-hFSH data sets found that disposition could be described using a two-compartment model and that absorption was rate limiting and essentially a first order process, for both compounds. The population estimate of clearance (CL) after i.v. administration was 0.60 and 0.44 l h⁻¹ for r-hFSH and u-hFSH respectively. The calculated mean residence times (MRT) for r-hFSH and u-hFSH were 16 and 18 h, respectively. The different bioavailabilities (F) and mean absorption times (MAT) determined after i.m. and s.c. administration ranged from 0.60 to 0.77 and from 27 h to 48 h, depending on compound, administration route, data type and method of analysis. Population analysis of the sparse patient data found that a one compartment model with first order absorption was adequate to describe the r-hFSH and u-hFSH data. The population estimates of apparent clearance (CL/F) were 0.71 and 0.33 l h⁻¹ for r-hFSH and u-hFSH respectively. Urinary-hFSH CL/F increased linearly with weight and was 0.33 l h⁻¹ at the average weight of 58.5 kg. No other covariates (age, weight, height, creatinine clearance, body mass index, race) were found to influence the FSH disposition parameters. The sparse data population estimates of intersubject variability in CL/F for r-hFSH and u-hFSH were essentially the same, 26% and 25%, respectively. Table 1 results in a calculated MRT of 16 h and a terminal half-life of 14 h.[23]

Table 1. Population pharmacokinetic parameter estimates for the typical individual after i.v. administration of 150 IU of r-hFSH and u-hFSH. The values in parentheses are the estimates of intersubject variability.[23]

<table>
<thead>
<tr>
<th>Population parameters</th>
<th>r-hFSH</th>
<th>u-hFSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance (L·h⁻¹)</td>
<td>0.60 (27%)</td>
<td>0.44 (12%)</td>
</tr>
<tr>
<td>Central volume (L)</td>
<td>5.34 (26%)</td>
<td>4.49 (9%)</td>
</tr>
<tr>
<td>Intercompartment clearance (L·h⁻¹)</td>
<td>0.58 (40%)</td>
<td>0.44 (22%)</td>
</tr>
<tr>
<td>Volume of distribution (L)</td>
<td>9.60 (27%)</td>
<td>7.74 (9%)</td>
</tr>
<tr>
<td>Baseline (IU·L⁻¹)</td>
<td>1.82 (29%)</td>
<td>2.18 (39%)</td>
</tr>
<tr>
<td>Baseline slope (IU·L⁻³/week)</td>
<td>0.22 (0.26)</td>
<td>-0.05 (0.61)</td>
</tr>
</tbody>
</table>
The population model prediction of the serum FSH profile after administration of r-hFSH to the typical individual, together with a 67% prediction interval is shown in Fig 3. [23]

The population analysis and disconsolation techniques of rich and sparse data sets have enabled the pharmacokinetics of r-hFSH and u-hFSH to be characterized in subjects undergoing IVF-ET treatment. The analyses demonstrated that absorption is the rate limiting step for both compounds and that none of the covariates tested influenced the disposition of r-hFSH. The population analysis indicates that the variability in CL/F is moderate, consequently, so would be the variability in exposure, given a fixed dosage regimen.[23]

Fig 3. The population model predicted FSH profile after administration of r-hFSH given subcutaneously (225 IU FSH once daily) to the typical individual and associated 67% prediction interval [23]

Pharmacokinetic / pharmacodynamic Study

As explained above, immunoassays only indicate the amount of intact FSH molecules present in the circulation and do not reflect the extent to which these molecules bind to the FSH receptor and are able to trigger target cells. In comparative pharmacokinetic studies of rFSH, in which equal doses of in vivo bioactive FSH were administered, the amount of immunoreactive FSH after a single dose of rFSH was significantly lower than after a comparable dose of urinary FSH. Whether differences in serum immunoreactive FSH concentrations are predictive for the magnitude of ovarian response depends on the bioactivity of circulating FSH glycoforms. The latter can only be measured by an in-vitro bioassay, which was also applied in the single-dose study of rFSH in gonadotrophin-deficient volunteers. For this purpose, serum samples taken prior to recombinant or urinary FSH injection, and taken 6, 24 and 72 h thereafter, were analysed in both the immunofluorometric assay and the in-vitro granulosa cell bioassay.[24] The calculated ratio of the outcome of these assays, called the B:I ratio, is presented in Table 2.

Table 2. Bio:immuno ratios of serum FSH in gonadotrophin-deficient subjects measured prior to injection and 6, 24 and 72 h after the injection of 300 IU rFSH and 300 IU urinary FSH[24]

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>gonadotrophin-deficient Men</th>
<th>gonadotrophin-deficient Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>rFSH (n=7)</td>
<td>u-FSH (n=4)</td>
<td>rFSH (n=7)</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>4.1 (2.2-5.5)</td>
<td>2.4 (1.4-3.5)</td>
</tr>
<tr>
<td>24</td>
<td>4.4 (3.1-6.6)</td>
<td>2.5 (ND-2.7)</td>
</tr>
<tr>
<td>72</td>
<td>5.5 (ND-21.0)</td>
<td>1.1 (ND-6.4)</td>
</tr>
</tbody>
</table>

In all subjects, endogenous bioactive FSH measured in pretreatment samples was under the detection limit of the bioassay (~3.7 IU/L). Individual B FSH concentrations measured at 6, 24 and 72 h after rFSH or urinary FSH administration were undetectable at all time points in one woman (body weight 85.8 kg) who was excluded from further evaluation. In all subjects treated with rFSH, B FSH was measurable for at least 72 h; during this period, no significant changes in the B:I ratio occurred. In addition, there was no significant difference in the B:I ratio between men and women. The B FSH concentrations after rFSH treatment tended to be higher than those after urinary FSH treatment, whereas the I FSH concentration showed an opposite tendency. As a result, in all post-treatment samples the B:I ratio was significantly higher after rFSH treatment than after urinary FSH treatment. This obviously clinically relevant difference will have to be confirmed in similar studies comprising larger
numbers of patients.\cite{16}

A daily dose of 75 IU rFSH, given for 7 days, appeared to be too low to induce significant follicular growth (diameter of at least 10 mm) in any of the nine volunteers. In contrast, follicular growth was induced in all other subjects treated with 150 or 225 IU rFSH or 150 IU urinary FSH. The total number and size of follicles induced by 150 IU rFSH and 150 IU urinary FSH are depicted in Fig 4.\cite{16}

Fig 4. Total number and size of follicles in pituitary-suppressed women during and after daily i.m. treatment with 150 IU r-hFSH; upper panel and 150 IU u-hFSH (lower panel).

After the administration of equal doses of in-vivo bioactive FSH, the amount of serum immunoreactive FSH might be significantly different between FSH preparations because of differences in their isohormone profiles. This may also explain why even though serum immunoreactive FSH concentrations are lower or equal, the actual bioactivity of this circulating FSH can be higher. Therefore, the conventional idea that serum immunoreactive FSH correlates positively with the magnitude of ovarian response should be reconsidered. This study is suggested that relatively basic isohormones, because of their higher intrinsic bioactivity, play an important role in the induction of multiple follicular growth. Therefore, the conventional idea that serum immunoreactive FSH correlates positively with the magnitude of the ovarian response should be reconsidered.\cite{16}

**Conclusion**

Follicle stimulating hormone (FSH) is a heterodimeric glycoprotein hormones secreted by anterior pituitary gland and is composed of two non-covalently linked protein subunits, the α-subunit which is common to other glycoprotein hormones, and the a hormone-specific β-subunit. FSH preparation plays an essential role in the treatment of human infertility. Recombinant human follicle stimulating hormone (r-hFSH) which is produced by recombinant DNA technology has the following characteristics: it is a urine-independent production process; good batch-to-batch consistency is ensured; effective purification takes place; absence of LH activity; and the final product is suitable for subcutaneous injection. Pharmacokinetics studies of single and repeated dose were carried out in clinical studies. A large number of PK studies provided scientific basis and value for reasonable application of the product.

**References**

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