Population pharmacokinetic modelling of Emfilermin (recombinant human leukaemia inhibitory factor, r-hLIF) in healthy postmenopausal women and in infertile patients undergoing *in vitro* fertilization and embryo transfer

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Keywords

leukaemia inhibitory factor (LIF), NONMEM, population pharmacokinetics

Received

25 August 2003

Accepted

2 December 2003

Aims

The aim of this analysis was to develop a population pharmacokinetic model for Emfilermin (recombinant human leukaemia inhibitory factor, r-hLIF) following subcutaneous administration to healthy postmenopausal women and to infertile patients undergoing *in vitro* fertilization and embryo transfer (IVF-ET).

Methods

Data from three studies, a single and a repeat dose Phase I study in postmenopausal women as well as a proof of concept study in patients undergoing *in vitro* fertilization and embryo transfer were combined and analyzed. The structural pharmacokinetic model was developed using the rich data from the Phase I studies and the full pharmacostatistical model was then derived using all the data.

Results

The pharmacokinetics of r-hLIF after repeated subcutaneous administration were described by a one-compartment disposition model with a zero order input. The duration of the absorption phase was short (0.8 h) and invariant. The apparent clearance in postmenopausal women was 57 l h⁻¹ (CV = 17%). In *in vitro* fertilization and embryo transfer patients, the apparent clearance was decreased by 35% compared with postmenopausal women. The apparent volume of distribution was 235 l (interindividual CV = 28%) and exhibited an interoccasion variability of 23%. It increased (for weight above 62 kg) or decreased (for weight below 62 kg) by 29% for every 10 kg body weight. The median *posthoc* estimates of apparent clearance and volume of distribution and their variability were consistent with the population estimates. In postmenopausal women, the results were consistent with those obtained by noncompartmental analysis. The residual variability on r-hLIF serum concentrations was 20%.

Conclusions

The pharmacokinetics of r-hLIF after repeated SC administration were described by a one compartment disposition model, with zero order input, in postmenopausal women and those undergoing IVF or intracytoplasmic sperm injection and embryo transfer. Absorption of r-hLIF was rapid as was its subsequent clearance. The apparent volume of distribution of r-hLIF was moderate to high, depended on body weight and showed interoccasion variability.

Introduction

Native leukaemia inhibitory factor (LIF) is a 38–62 kDa, heavily glycosylated, pleiotropic cytokine that belongs to the IL-6 family [1]. Named for its ability to inhibit proliferation of a myeloid leukaemia cell line by inducing differentiation, LIF also regulates the growth and differentiation of numerous other cells. LIF is amongst the mediators postulated to be involved in embryonic development and implantation [2, 3], the latter role being demonstrated first using a knock-out mouse model [4–6] with additional supportive evidence from *in vitro* [7] and *in vivo* data in animals using anti-LIF antibodies [8–10].

In humans, both the mature oocyte and preimplantation blastocyst express an LIF receptor, supporting the hypothesis that a specific interaction between the embryo and the maternal reproductive system precedes the penetration of the endometrium by the developing blastocysts [11, 12]. Cytotrophoblasts cultured in medium containing LIF were shown to differentiate towards the anchoring extravillous phenotype [13], and embryos cultured in LIF-containing medium showed an enhanced blastocyst formation rate and improved embryo quality [14, 15]. There is circumstantial evidence that a dysfunction of LIF expression contributes to human infertility and may be a cause of recurrent failure of implantation [16, 17–26]. However, a direct link between LIF and pregnancy has yet to be confirmed [27, 28], related in part to limitations in methods used to measure LIF [29, Young *et al.*, personal communication].

A nonglycosylated human LIF produced by a recombinant DNA technique (r-hLIF, Emfilermin INN, AMRAD Corp. Ltd, Australia (MWt *circa* 20 kDa)) was shown to possess biological activity (30). Its effect on embryo implantation in women with recurrent failure of assisted reproductive technology has recently been evaluated in a proof of concept study [31]. The object of the present work was to develop a population pharmacokinetic model for Emfilermin following subcutaneous administration. A structural model was developed using rich data sets from two Phase I studies in postmenopausal women. The final pharmacokinetic model was then developed pooling these data with sparse data from the above mentioned proof of concept study in younger infertile women.

Methods

Subjects

A total of 64 subjects from three studies were included in the analysis:

Study 1: Fourteen healthy postmenopausal women from a phase I single dose study

Study 2: Eleven healthy postmenopausal women from a phase I repeated dose study

Study 3: Thirty-nine premenopausal women with recurrent implantation failure justifying *in vitro* fertilization and embryo transfer from the proof of concept study.

Characteristics of the subjects, assessed at baseline, that were considered as potential covariates are summarized in Table 1. Age and population ‘type’ were essentially confounded since the phase I program was conducted in healthy but older postmenopausal women and the target population consisted of younger women with recurrent implantation failure undergoing *in vitro* fertilization and embryo transfer. All other potential covariates were well balanced across the three studies and the two populations.

All subjects gave informed consent to participate. Study 1 was approved by the Cambridge Local Research Ethics Committee, Addenbrooke’s Hospital, Cambridge, UK, and studies 2 and 3 by the Huntington Local Research Ethics Committee, Papworth Hospital, Cambridge, UK. Study 3 was also approved by the CARE at the Park Ethics Committee, The Park Hospital, Nottingham, UK.

Study design

Study 1 was a single-blind, randomized, parallel group, phase I design. Postmenopausal female subjects receiving hormone replacement therapy for the menopause received single 100 µg or 250 µg subcutaneous doses of Emfilermin. Subjects received two cycles of hormone replacement therapy separated by a period of 5 days as follows:

21 days of Cyclo-progynova® (combination of oestradiol and norgestrel)

14 days of ascending doses of Progynova® (oral oestradiol valerate) and from days 15–23 of this cycle, a fixed dose (600 mg day⁻¹) of Utrogestan® (oral micronized progesterone).

On day 22 of the second cycle, a single dose of either 100 µg or 250 µg Emfilermin was administered SC. Blood samples for measurement of serum LIF concentrations were collected at the following times: predose, 30 min, 1, 2, 3, 4, 8, 12 and 24 h postdose.

Study 2 was a double-blind, randomized, placebo controlled phase I design. Postmenopausal female subjects received two cycles of hormone replacement therapy separated by a period of 7 days as follows:

Table 1

Subject characteristics by study

Protocol	Variable	Median	Range (min-max)
Study 1 (<i>n</i> = 14) Phase I single dose Postmenopausal healthy women	Age (years)	58	50–64
	Weight (kg)	63	55–70
	ALT (IU l ⁻¹)	16	6–21
	AST (IU l ⁻¹)	20	15–30
	Creatinine (μmol l ⁻¹)	80	66–90
	Total bilirubin (μmol l ⁻¹)	8.7	5.6–13.9
Study 2 (<i>n</i> = 11) Phase I repeated dose Postmenopausal healthy women	Age (years)	58	49–63
	Weight (kg)	63	56–80
	ALT (IU l ⁻¹)	19	7–29
	AST (IU l ⁻¹)	24	15–33
	Creatinine (μmol l ⁻¹)	77	62–80
	Total bilirubin (μmol l ⁻¹)	12.3	8.0–19.0
Study 3 (<i>n</i> = 39) Proof of concept Women undergoing IVF-ET	Age (years)	34	30–37
	Weight (kg)	61	48–83
	ALT (IU l ⁻¹)	13	7–26
	AST (IU l ⁻¹)	17	11–29
	Creatinine (μmol l ⁻¹)	77	55–103
	Total bilirubin (μmol l ⁻¹)	9.7	3.7–16.9

21 days of Cyclo-progynova® (combination of oestradiol and norgestrel)

26 days of ascending doses of Progynova® (oral oestradiol valerate) and from days 15–26 of this cycle, a fixed dose (600 mg day⁻¹) of Utrogestan® (oral micronized progesterone).

The first day of administration of Progynova® was defined as study day 1. Subjects received 150 μg of Emfilermin or placebo SC twice daily from study day 17 for 7 days. Blood samples for pharmacokinetics (PK day 1), corresponded to the first day of Emfilermin administration and subsequent pharmacokinetic days were labelled relative to PK day 1. Blood samples for measurement of serum LIF concentrations were collected at the following times:

Morning dose, PK day 1: predose, 30 min, 1, 2, 3, 4, 8 and 12 h postdose

Morning dose, PK days 2–6: one predose sample on each day

PK day 7: predose, 30 min, 1, 2, 3, 4, 8 and 12 h postdose
Evening dose, PK day 7: 12, 16 and 24 h postdose.

Study 3 was a prospective, two-centre, randomized, double-blind, placebo-controlled, proof of concept design. Patients were young infertile women justifying *in vitro* fertilization or intracytoplasmic sperm injection and embryo transfer with a previous history of at least three implantation failures and no other known cause for failure of assisted reproductive technology. Nafarelin

(Synarel®) was self-administered twice daily by the intranasal route. The starting dose was 800 μg day⁻¹ (two sprays twice daily) until pituitary down-regulation was confirmed based on ovarian ultrasound scan and measurement of serum oestradiol concentrations. The dose of nafarelin was reduced to 400 μg day⁻¹ (one spray twice daily) on the first day of follicular stimulation (S1). Treatment with recombinant-human follicle stimulating hormone (r-hFSH, Gonal-f®) began once pituitary desensitization had been confirmed and this day was labelled S1 for each patient. On the morning of day 6 (S6), an ultrasound scan for evaluation of ovarian activity and endometrial thickness was performed and serum oestradiol (E₂) concentrations were measured to confirm pituitary desensitization. The dose of r-hFSH was adjusted on the same day according to the individual ovarian response and the normal practice of the centre. A single injection of 250 μg of recombinant human chorionic gonadotropin (r-hCG, Ovitrille®) was then administered to induce final follicular maturation within 36 h of the last r-hFSH injection. Oocytes were retrieved 34–38 h after r-hCG administration. Patients were randomized on the day of embryo transfer to either 150 μg Emfilermin twice daily or placebo for 7 days by the SC route of administration. PK day 1 corresponded to the first day of Emfilermin administration and subsequent PK days were labelled relative to that day. Blood samples for measurement of serum LIF concentrations were collected at the following times:

PK day 1: predose and 1–2 h postdose (two samples)
 PK day 4: 2–4 h postdose (one sample)
 PK day 7: 2–4 h postdose (one sample)

Exclusion of data and data set analysed

In studies 2 and 3 serum LIF concentrations from the placebo group were all below the limit of quantification of the assay. All samples from the active treatment groups that were below the limit of quantification of the assay were treated as missing in the data analysis. Subject 113 in study 1, and receiving 100 µg of Emfilermin, had only three measurable concentrations, which were only slightly above the limit of quantification. These concentrations were much lower than those in all other subjects in that group and a dosing error was suspected. Subject 116 in study 2 did not have peripheral veins that easily allowed the drawing of blood, and most samples from this subject were haemolyzed. Data from these two subjects were excluded from the analysis, and the data set analyzed consisted of 342 samples from 64 subjects (226 samples from 25 healthy postmenopausal women and 116 samples from 39 patients).

Analysis of r-hLIF

A commercially available ELISA (R & D Systems, Minneapolis, USA) was used to measure r-hLIF concentrations following internal validation (data on file). The following validation data were obtained: standard range: 31.2–2000 pg ml⁻¹; limit of quantification: 50 pg ml⁻¹; limit of detection: ≤ 8 pg ml⁻¹; intra-assay precision: 5.7–8.8%; interassay precision: 2.4–6.1%; and accuracy: 86–110%. The assay has a cross-reactivity of 1–7% with endogenous human LIF.

Population pharmacokinetic model development

A population pharmacokinetic (PK) model was developed and fitted to the LIF concentration data using NONMEM V version 1.1 and Visual NONMEM on a PC with a Windows 2000 operating system. A Digital Visual Fortran 5.0 compiler was used. In NONMEM a First Order Conditional Estimation method (FOCE) was used for minimization [32].

Step 1: Basic pharmacokinetic and statistical models

The base pharmacokinetic model was defined as the model that best described the mean population and individual tendencies, in the absence of any covariate effects.

For the base model, the observations are expressed as follows [32]:

$$\text{OBS}_{ij} = f(\theta_i, D, t_j) + \varepsilon_{ij}$$

in which OBS_{ij} refers to the j^{th} observation (serum LIF concentrations) in the i^{th} individual, f represents the structural pharmacokinetic model, θ_i represents the set of pharmacokinetic parameters for the i^{th} individual, D is the dosing regimen administered to that individual, t_j represents the independent variable time and ε_{ij} represents the residual shift of the observations from the model predictions. For the analysis ε_{ij} 's are assumed to be random variables normally distributed around zero, with variance of σ^2 .

The disposition of r-hLIF was adequately described by a one-compartment open model defined in terms of apparent clearance (CL/F) and apparent volume of distribution (V/F). Zero, first, and a mixed parallel first and zero order absorption, with the dose fractionated between them, was explored, and the zero order input was selected as best describing the data.

Unexplained intersubject variability in the structural model parameters was modelled, assuming a log normal distribution η_i as follows:

$$P_i = \text{TVP} \exp(\eta_i)$$

where TVP is the typical value of the pharmacokinetic parameter P in the population, P_i is the individual value for P in the i^{th} individual and η_i is a random variable with a mean of zero and variance ω^2_P . Estimates of intersubject variability in P are represented as the square root of ω^2_P , which is an approximation of the coefficient of variation of P for a log-normally distributed quantity.

Random residual variability was modelled according to a proportional error model

$$C_{ij} = C_{ij}^* (1 + \varepsilon_{ij})$$

where C_{ij} is the i^{th} serum concentration measured in the i^{th} individual, C_{ij}^* is the respective model predicted concentration, and the ε_{ij} is the normally distributed error term with mean of zero and variance σ^2 . Additive error and combined additive and proportional error models were also explored.

Interoccasion variability (IOV) was evaluated as an additional level of random effect, expressed as follows:

$$P_i = \text{TVP} \exp(\text{IOV})$$

where

$$\text{IOV} = \kappa_{1i} \cdot \text{OCC}_1 + \kappa_{2i} \cdot \text{OCC}_2 + \dots + \kappa_{ni} \cdot \text{OCC}_n$$

OCC_n has the value of 1 for the n^{th} occasion and 0 otherwise, for each individual. $\kappa_{1...n}$ -values are random variables assumed to be normally distributed around zero with identical variance denoted by π^2_P [33].

In this analysis three occasions were defined: occasion 1, the first day of treatment; occasion 3, the last day

of treatment (either day 6 or 7); and occasion 2, all intermediate days (mostly day 4). Interoccasion variability was tested on CL/F and V/F as well as on both parameters together. Since, in general, there was only one measured r-hLIF concentration per subject per occasion in study 3, most of the information on IOV is derived from study 2.

Step 2. Covariate model: Univariate analysis

Covariate model building was performed within NONMEM [34].

Type of population (postmenopausal women or *in vitro* fertilization and embryo transfer women), dose, body weight, aspartate amino transferase (AST), alanine amino transferase (ALT), creatinine and bilirubin were examined as factors with potential influence on CL/F and V/F of LIF. To describe the relationship between continuous covariates (i.e. body weight, AST, ALT, creatinine, bilirubin) and a pharmacokinetic model parameter P , a linear model was assumed. Taking the covariate body weight (WGT) as an example, this model would be:

$$TVP = P_{pop} + \theta_{WGT} (WGT - ref_WGT)$$

where TVP is the model predicted value for P given the covariate value for WGT; P_{pop} represents the population central tendency for the pharmacokinetic parameter P , and corresponds to TVP evaluated at the reference value 'ref_WGT' for weight; θ_{WGT} represents the slope of the relationship. For subjects with weight above the reference value ref_WGT, TVP will be greater than P_{pop} , for subjects with weight below the reference value ref_WGT, TVP will be lower than P_{pop} .

As reference value for the selected covariates, the median value in the population investigated was used: body weight = 62 kg, ALT = 15 IU l⁻¹, AST = 18 IU l⁻¹, creatinine = 77.5 µmol l⁻¹, bilirubin = 10 µmol l⁻¹.

Relationships between categorical covariates (e.g. type of population) and a pharmacokinetic model parameter P was modelled as follows:

$$TVP = P_{pop} \quad \text{for postmenopausal women}$$

$$TVP = P_{pop} \times \theta_{TYPE} \quad \text{for } in\ vitro \text{ fertilization and embryo transfer women}$$

P_{pop} corresponds to TVP for the reference covariate value, which was postmenopausal women for type of population. The effect of type of population is quantified by θ_{TYPE} giving the relative change in TVP of young patients with respect to healthy postmenopausal subjects.

Possible relationships between the pharmacokinetic parameters and each covariate were evaluated using

univariate analysis in NONMEM. All covariates were ranked by size of effect on the objective function (OBJ) by comparison to the base model (largest decrease was rank 1).

Step 3. Covariate model selection

Only covariates that decreased the OBJ by more than 7.9 (i.e. $P < 0.005$) when compared with the base model were considered for the full covariate selection. Model 2 consisted of the model with the single covariate that gave the largest drop in the OBJ relative to the base model. Backward addition was then performed from model 2 by adding the covariates one at a time with the lowest rank first, and by fitting to the data, resulting in a new OBJ. If the improvement in OBJ was ≥ 7.9 (at a risk level of 0.005), the covariate was retained. The model resulting from this backward addition procedure was labelled model 3.

Forward deletion was then performed by removing one covariate at a time from model 3 to test if the improvement in OBJ was ≥ 7.9 . Covariates, that produced this magnitude of increase, were retained, and other covariates were removed from the model. The outcome of this procedure was the final model. In addition to the change in OBJ, diagnostic plots were examined at each model-building step.

Results

The choice of the structural model was based on the rich data only. Diagnostic plots indicated that the zero order absorption model fitted the data better than the first order absorption and the combined parallel zero-and-first-order absorption models. The base population model included interindividual variability terms on two fixed-effect parameters, apparent clearance (CL/F) and apparent volume of distribution (V/F). The estimated intersubject variability in the duration of input (D1) was very small (10^{-9}) and thus it was omitted from the base model.

With respect to residual variability, an additive error model did not fit the data. The combined (proportional and additive) error model provided a poor estimate of the additive component with a large 95% confidence interval spanning zero. Therefore residual error was modelled using a proportional error model.

Inter-occasion variability was tested on CL/F and V/F . Introduction of IOV on CL/F in the model provided a decrease of 21 points in the OBJ. The CV associated with IOV was 14% and the corresponding 95% confidence interval spanned zero. Introduction of IOV on V/F in the model provided a decrease of 30 points in the OBJ. The CV was 25% and the 95% confidence interval

indicated that the IOV on V/F was precisely estimated. Based on this it was decided to retain IOV only on V/F in the base pharmacokinetic and statistical model, for which parameter estimates are presented in Table 2.

Each single combination of a covariate with one of the pharmacokinetic parameters (CL/F and V/F) (Table 3) was included sequentially into the base model.

Covariates that could potentially influence the estimates of one or both pharmacokinetic parameters at the significance level of $P < 0.005$ were: dose, 'type' of population and body weight. The largest effect found was 'type' of population with patients (*in vitro* fertilization and embryo transfer) having a lower apparent clearance compared with healthy postmenopausal women. The model including this covariate effect was labelled model 2. Following backward addition and forward deletion, two effects remained in the model, namely those of 'type' of population on CL/F and body weight on V/F .

As the phase I program was conducted in healthy but older postmenopausal women and the proof of concept study in young patients undergoing *in vitro* fertilization and embryo transfer the covariates *age* and 'type' of population were essentially confounded. Therefore, the analysis was repeated including *age* as a covariate and omitting population 'type'. As a result the decrease in OBJ with respect to *age* when included as a single covariate on CL/F was only 3.5 points, and it was not retained in the final model. Therefore inclusion of population 'type' rather than *age* in the analysis appears to be justified. *Study type* was also confounded by *age* and 'type' of population. However, as the same batch of r-hLIF was used in all three studies, there was no *a priori* reason to include study type as a covariate in the analysis.

Table 2

Model parameters (base model) including inter-occasion variability

Parameter	TVP	ISV	IOV	Remark
CL/F ($l\ h^{-1}$)	53.4	24%	–	
V/F (l)	199	39%	25%	IOV on V/F
D1 (h)	1.07	–	–	No ISV estimated
RV		19%		

TVP: typical value; ISV: intersubject variability; IOV: inter-occasion variability; RV: residual variability. ISV, IOV and RV expressed as % CV: coefficient of variation (%).

Figure 1 and Tables 4 and 5 summarize the results obtained with the final population pharmacokinetic model. Figure 2 shows the observed and predicted serum LIF concentrations in three subjects from each study. All parameters were well estimated ($CV < 30\%$ and confidence intervals did not span zero). No particular trend was detected in the diagnostic plots (see Figure 1). Inter-subject variability was decreased in the final model compared with the base model, and residual error remained at the same level. Duration of absorption was estimated at 0.8 h (intersubject variability was not determined for this parameter). For healthy postmenopausal women, the typical value for CL/F was $57\ l\ h^{-1}$, which was decreased by 35% (i.e. $37\ l\ h^{-1}$) in *in vitro* fertilization and embryo transfer patients. The intersubject variability in CL/F was decreased from 24% in the base model to 17% in the final model, indicating that population 'type' partially explains the variability in CL/F .

The existence of an interoccasion variability was demonstrated for the apparent volume of distribution (CV of 23%). The population value for the apparent volume of distribution was 235 l for a median body weight of 62 kg. V/F increased for weight above 62 kg and decreased for weight below 62 kg by 29% every 10 kg. The intersubject variability for V/F was decreased from 39% in the base model to 28% in the final model, indicating that body weight partially explains the variability in V/F .

The terminal half-life estimated as a secondary parameter was 2.9 h for postmenopausal women and

Table 3

Decreases in NONMEM objective function values after inclusion of single covariate/PK parameter pairs into the base model

Covariate	Pharmacokinetic parameter	
	CL/F	V/F
Dose group	0.72 (NS)	12.4 (NR)
Type (healthy/patient)	33.4 (+)	16.8 (NR)
Weight	18.2 (NR)	14.1 (+)
ALT	0.01 (NS)	0.93 (NS)
AST	0.03 (NS)	0.89 (NS)
Creatinine	2.57 (NS)	1.49 (NS)
Bilirubin	1.26 (NS)	0.32 (NS)

NS not significant (Δ objective function < 7.9); + effect included in final population model; NR not retained in final population model.

Figure 1

Final population pharmacokinetic model: diagnostic plots for model predictions. a: Predicted vs observed concentrations (pg ml^{-1}) (solid line: line of identity). b: Weighted residuals vs predicted concentrations. c: Weighted residuals vs time. d: Individual predicted vs observed concentrations (pg ml^{-1}) (solid line: line of identity)

Table 4

Final population pharmacokinetics model estimates

Parameter	TVP (SE)	ISV (SE)	IOV (SE)	Remark
CL/F (L/h)	57.0 (2.81)	17 (0.0112)	–	
V/F (L)	235 (14)	28 (0.0342)	23 (0.0178)	IOV on V/F
D1 (h)	0.84 (0.0431)	–	–	No ISV estimated
Type on CL/F	0.649 (0.0454)			Effect of Type on CL/F
Weight on V/F	6.7 (1.65)			Effect of Weight on V/F
RV		20 (0.0046)		

TVP typical value; ISV intersubject variability; IOV interoccasion variability; RV residual variability; SE standard error. ISV, IOV and RV expressed as % CV coefficient of variation (%).

4.4 h for women undergoing *in vitro* fertilization and embryo transfer. The residual error remained stable at 20% CV.

Table 6 presents the summary statistics of V/F by study by occasion. For the proof of concept study interoccasion variability in this parameter was random. However, there was a systematic decrease in V/F in study 2 suggesting possible time dependence in the pharmacokinetics of r-hLIF in healthy postmenopausal women.

Discussion

The present work describes the pharmacokinetics of r-hLIF after SC administration in healthy postmenopausal women and younger patients undergoing *in vitro* fertilization and embryo transfer. The pharmacokinetics of r-hLIF were best described by a one-compartment disposition model, with zero-order input from the subcutaneous tissue. Absorption was very rapid and invariant. Recombinant-hLIF was rapidly cleared from the body. In younger patients undergoing *in vitro* fertilization and embryo transfer, the apparent clearance was 35% lower than in older postmenopausal women. Apparent volume of distribution (V/F) was relatively large at 235 l (CV 28%), and was linearly related to the body weight. V/F increased (for weight above 62 kg) or decreased (for weight below 62 kg) by 29% every 10 kg. For all parameters the population estimates were consistent with the median Bayesian *post hoc* estimates and for older postmenopausal women were close to the median values derived from noncompartmental analysis (data not shown).

The 35% decrease in apparent clearance in younger women undergoing *in vitro* fertilization and embryo transfer compared with healthy older postmenopausal women is an unexpected finding. Women undergoing *in vitro* fertilization and embryo transfer receive r-hFSH to stimulate the ovarian response, whereas the healthy postmenopausal women do not. Although unlikely on theoretical grounds, inhibition of r-hLIF metabolism by r-hFSH, resulting in decreased clearance of the former, cannot be excluded. Furthermore, the marked difference in hormonal status and physiology between postmenopausal women on hormone replacement therapy and younger women undergoing controlled ovarian stimulation for *in vitro* fertilization could induce changes in mediators of r-hLIF clearance (such as, putatively, LIF

Table 5

Statistically significant covariate effects in the final population pharmacokinetic model on the pharmacokinetic parameters

Statistically significant effect	Effect on the PK parameter	Remark
Effect of type of population on CL/F	CL/F 35% lower in IVF-ET women compared with PM women	PM women: CL/F = 57 l h ⁻¹ IVF-ET women: CL/F = 37 l h ⁻¹
Effect of body weight on V/F	V/F increased (for weight above 62 kg) or decreased (for weight below 62 kg) by 29% every 10 kg	For weight = 62 kg, V/F = 235 l

IVF-ET in vitro fertilization and embryo transfer; PM postmenopausal. **Figure 2**

Serum LIF concentration profiles for three subjects from each study. Open circles represent the individual measured concentrations; solid lines and stars represent the individual predictions obtained with the final pharmacokinetic model. The subjects shown in Figure 2 are representative of the population (i.e. at the median of the population based on their average weighted residuals)

receptors). Finally, the exact dose of r-hLIF, administered in the two phase I studies was known, as the syringes were weighed before and after it was given. However, in the proof of concept study the *nominal* dose had to be used since syringes were not weighed. A bias

in the nominal dose relative to the actual dose could explain the difference in apparent clearance between the populations. In the univariate analysis, a similar effect was seen in *V/F*. However, this effect was eliminated during model building and did not appear in the final

Table 6

Post hoc estimates of apparent volume of distribution by study and by occasion

Study	Occasion	n	Median (l)	Range (min-max) (l)
1	1	14	303	181–505
2	1	11	288	153–711
	2	2	294	290–299
	3	9	189	116–536
3	1	39	229	98–358
	2	39	229	108–376
	3	39	228	109–365

model. In order to assess the influence of using the actual vs nominal dose on outcome, the analysis was repeated using the nominal dose administered to all subjects. In this alternative analysis the 'type' of population had no significant effect on apparent clearance. Therefore we suspect that younger subjects have a higher bioavailability and/or higher than intended doses were administered in the proof of concept study. To confirm linearity, as implied by the structural pharmacokinetic model, dose was included as a potential covariate during the analysis and proved not to be statistically significant in the range 100–250 µg.

In analyzing the results of study 2 using noncompartmental analysis (data not shown) an unexpected, given the short half-life and dosing interval, accumulation of r-hLIF was seen on day 7 of treatment. Data from this study were well described by the present model despite apparent time dependence in the pharmacokinetics, owing to a systematic decrease in V/F (by 34% on average) from day 1 to day 7. An attempt was made to explicitly model this apparent time dependence allowing V/F to change from its initial value to a given target value, with the rate of change governed by a first order rate constant. However, the attempt failed possibly due to the fact that the apparent time dependence occurred only in study 2. The reason for the occurrence of apparent time dependence in V/F in healthy postmenopausal women but not in younger women undergoing *in vitro* fertilization and embryo transfer is not known. However, a systematic error in dosing can be excluded, since the exact doses administered in studies 1 and 2 were used in this analysis. This leaves an increase in bioavailability, a decrease in volume of distribution or a combination of the two as explanations for this time dependent phenomenon. A study involving IV and chronic (1 week) SC administration of r-hLIF would be neces-

sary to elucidate further the phenomenon but since it was not present in the target population, time dependence in V/F clearly has no clinical relevance.

In conclusion, the pharmacokinetics of r-hLIF after repeated SC administration were described by a one compartment disposition model, with zero order input, in postmenopausal women and those undergoing *in vitro* fertilization or intracytoplasmic sperm injection and embryo transfer. Absorption of r-hLIF was rapid as was its subsequent clearance. The apparent volume of distribution which was moderate to high, depended on body weight, and showed an interoccasion variability.

This work was sponsored by Serono International S.A.

The authors wish to acknowledge the kind assistance of the personnel of the Bourn Hall Clinic, Bourn, Cambridge, UK who ran the two phase I studies and, in part, the proof of concept study, the kind assistance of the personnel of CARE Park Hospital at Nottingham, UK (who ran, in part, the proof of concept study) and the Laboratory Unit in Bourn (Helen Young) who performed the bioanalytical work. The monitoring work of M. von Holtey and S. Lancaster is also greatly appreciated.

Emfilermin is licensed by AMRAD Corp. Ltd, Australia.

Conflict of interest: The authors are all employees of Serono International SA who are developing Emfilermin for use in reproduction.

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