Population Pharmacokinetic/ Pharmacodynamic Modeling of Cetrorelix, a Novel LH-RH Antagonist, and Testosterone in Rats and Dogs

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Received September 6, 1999; accepted December 7, 1999

Purpose. Population models for the pharmacokinetic-pharmacodynamic relationship for cetrorelix (CET), a luteinising hormone-releasing hormone (LH-RH) antagonist, and the pharmacodynamic response on testosterone production were investigated in rats and dogs.

Methods. The plasma concentrations of CET and testosterone were determined after intravenous and subcutaneous injections. The population PK/PD-models were developed using P-PHARM software.

Results. Absolute bioavailability of cetrorelix was 100% in rats and 97% in dogs. In rats, the pharmacokinetics was explained by a two-compartment model with saturable absorption, while a three-compartment model was used in dogs. Testosterone suppression in both species was described by a sigmoid E_{max} model with maximum effect (E_{max}) considered as total hormonal suppression. The duration of testosterone suppression in rats was longer at higher doses. The population elimination half-lifes after iv-dose were 3.0 h in rats and 9.3 h in dogs. Population mean estimates of IC_{50} were 1.39 and 1.24 ng/ml in rats and dogs, respectively.

Conclusions. A population pharmacokinetic model was developed to explain the dissolution rate limited absorption from the injection site. The suppression of testosterone could be described by an indirect inhibitory sigmoid E_{max} model. In both species 1–2 ng/ml CET in plasma was necessary to suppress testosterone production.

KEY WORDS: luteinising hormone-releasing hormone (LH-RH) antagonist; cetrorelix; pharmacokinetics; population PK/PD-modeling; testosterone; rat; dog.

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ABBREVIATIONS: CET, cetrorelix; PK/PD-modeling, pharmacokinetic/pharmacodynamic-modeling; RIA, radioimmunoassay; EIA, enzyme immunoassay; C_{obs}, C_{exp}, observed / expected drug concentration; k_{12}, k_{21}, k_{13}, k_{31}, k_{10}, k_a, pharmacokinetic hybrid constants; X_a, X_c, X_p, amount of drug at the injection site (a), in the central (c) or the peripheral (p) compartment; R_{max}, maximum dissolution rate; R_m, amount of cetrorelix at which the dissolution rate is 50% of the maximum; M, fraction of dose dissolved; D_0, amount of cetrorelix in solution at injection site; T, testosterone concentration; T_0, testosterone baseline; k_e, testosterone elimination rate constant; IC_{50}, CET concentration producing 50% of the maximum effect; E_{max}, maximum effect; n, Hill factor; P_{exp}, P_{pop}, expected individual/population parameter; SPPE, standardised parameter prediction error; SCPE, standardised concentration prediction error.

INTRODUCTION

Two classes of luteinising hormone-releasing hormone (LH-RH) analogues, agonists and antagonists, are under development for identical indications such as endometriosis and sex hormone-sensitive tumors. LH-RH antagonists inhibit LH-RH competitively depending on their concentration in the systemic circulation and therefore, cause an immediate suppression of LH, FSH, and thereby the sex hormone release, avoiding the characteristic initial surge of the hormone levels caused by LH-RH agonists. This relationship can be characterised by pharmacokinetic/pharmacodynamic (PK/PD) modeling. Until now, only little is known about PK/PD relationships between LH-RH antagonists and testosterone in rats and dogs. Even the release patterns of testosterone in both species are not well characterised. The release pattern of LH-RH is known to be pulsatile (1). However, the endogenous release pattern of testosterone in rat and dog still remains unclear. A circadian rhythm in dogs is controversially discussed (2,3).

The decapeptide cetrorelix (CET) has been characterised as a potent LH-RH receptor antagonist free of edematogenic effects (4). CET inhibits gonadotropin secretion by competing with endogenous LH-RH. This in consequence leads to suppression of testosterone production, resulting in suppression of the free testosterone plasma concentration in males. Some pharmacokinetic data after single doses in rats reported previously (5) were in good correlation with published data on pharmacokinetics of LH-RH and its analogues (6–15, Table I). In addition to the plasma levels of CET in male animals, testosterone concentrations were also determined. Testosterone suppression was followed in male rats and dogs to determine the duration of action. It was the objective of this paper to identify a population PK/PD-model linking CET plasma concentrations after iv- and sc-administrations in rats and dogs to the pharmacodynamic response of testosterone suppression.

MATERIALS AND METHODS

Compound

Cetrorelix acetate salt (CET; [Ac-D-Nal(2)[1], D-Phe(4Cl)[2], D-Pal(3)[3], D-Cit[6], D-Ala[10]]LH-RH) was synthesised by ASTA Medica AG, Frankfurt, Germany. Two different [^{14}C]labelled batches of CET were used in the dog studies. Precursor peptides were synthesised by Degussa AG (Hanau, Germany) and uniformly labelled amino acids were introduced by Amersham International (Little Chalfont, England).

Animals

Male and female albino Wistar rats (WIGA, Sülzfeld, Germany) weighing between 270–350 g and male and female Beagle dogs (Harlan Winkelmann, Borchen, Germany) weighing between 11.5–14.5 kg were used. The animals were acclimatised at a temperature of 20–22°C and a relative humidity of 50–55% under natural light/dark conditions for at minimum 1 week before dosing.

Study Design

CET was administered to groups of 4 male and 4 female rats or to groups of 3–4 male and female dogs in a 5.2%

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Table I. Elimination Half-Lives of CET in Comparison to LH-RH and Other LH-RH Analogues Following a Single Intravenous Injection to Rats, Dogs or Monkeys at Different Dose Levels

	Rat		Dog		Monkey		
Peptide	Dose [mg/kg]	$t_{1/2}$ [h]	Dose [mg/kg]	$t_{1/2}$ [h]	Dose [mg/kg]	$t_{1/2}$ [h]	Reference
LH-RH	0.043	0.2					6
	0.46	0.1			0.008	0.6	7
LH-RH agonists (D-aminoacid in position 6)							
[D-Phe^6]LH-RH	0.025	0.4					8
Triptorelin			0.1	1.7			9
Leuprorelin	0.1	0.6[a]	0.1	1.5[a]			10
Meterelin			0.01	1.8			11
Nafarelin	0.53	0.6			0.07	2	7
LH-RH antagonists (D-aminoacids in positions 1, 2, 3, 6 and 10)							
A-75998	0.1	1.14	0.03	7.99	0.03	5.75	12
Antarelix			0.01	11			13
Cetrorelix	0.1	1.7[b]	0.1	8.7[b]			5 (rat)
Detirelix	0.3	1.6			0.08	7	14
Ganirelix	1	1.4			1	10	15

[a] Extrapolation performed from the data reported.
[b] Values from non-compartmental data analysis.

mannitol solution intravenously at a dose of 0.1 mg/kg and in a volume of 1 ml/kg. Subcutaneous injection was performed in rats at doses of 0.02, 0.1, 0.25 and 0.5 mg/kg with a constant volume of 1 ml/kg in all dose levels and in dogs at a dose of 0.1 mg/kg in a volume of 0.5 ml/kg. Additionally a control group of male rats was administered subcutaneously with vehicle only (5.2% mannitol solution, 1 ml/kg) for determination of testosterone plasma concentrations under testing conditions. Blood from rats was collected repetitively by puncture of the ophthalmic venous plexus with heparinised micro-hematocrit tubes. In dogs, blood was obtained from the vena cephalica antebrachii of one foreleg (in the iv-administered groups the one not used for administration) by using NH_4-heparinised syringes (Monovetten, Sarstedt, Nümbrecht, Germany) and plasma was separated by centrifugation (10 min, 2,000*g*). All animals were housed individually during the study with free access to standard diet and water.

Bioanalystics

CET plasma concentrations were analysed by a specific radioimmunoassay (16). All determinations (calibration, QC standards and samples) were performed in triplicate (3 × 20 μL plasma). Polyclonal CET antiserum from rabbit, [^{125}J]CET and rat plasma were incubated in RIA buffer for 2 days at 4°C. The separation of free and antibody-bound [^{125}J]CET was achieved after addition of rabbit IgG, anti-rabbit IgG (goat) and polyethylene glycol following centrifugation at 2,000× g for 20 min at 4°C. The lower limit of quantitation ranged from 0.1 to 0.4 ng/ml in the different batches. The acceptance criteria for each RIA batch was 4 of 6 QC standards (0.5, 2.5 and 5 ng/ml) had to show an accuracy of <25% CV and (maximal) 2 outliers should not be of the same concentration. Validation for measurement in rat plasma resulted in an accuracy of 87.2–103.4% and a precision of 3.1–8.5%, for dog plasma accuracy was 99–111.4% and precision 3.1–16%. Samples with concentrations above the calibration range were diluted and reanalysed. The RIA method was cross-validated using an HPLC method (17).

Testosterone plasma concentrations were analysed by a commercially available specific enzyme Immunoassay (DRG Instruments, Marburg, Germany). The EIA is based on a competitive binding of free testosterone in the sample versus a fixed amount of testosterone enzyme conjugate to a specific testosterone antibody bound on the microtiter plate test-wells. The determinations were performed in duplicate (2 × 50 μl plasma). Calibration was performed with lyophilised standards (0.15–15.7 ng/ml). QC standards (low: 0.54 or 0.58 ng/ml and high: 2 or 2.3 ng/ml) were supplied together with each assay. The assay was validated for the determination of testosterone in rat and dog plasma. Accuracy during validation was 92.2–121.1%, precision was 8.2–23.8%.

Data Analysis

Noncompartmental evaluation of the pharmacokinetic parameters from the plasma concentration-time profiles for all individuals was performed using Excel 5.0 (Microsoft Corporation, Seattle, USA). The area under the plasma time curve (AUC) was calculated by the linear-trapezoidal rule. The mean residence time (MRT) was calculated as the ratio from the first moment curve (AUMC) and AUC and the total plasma clearance (CL) as the ratio of dose and AUC.

In rats, AUC values showed dose-proportionality while C_{max} values did not show linear relationship with dose. Terminal elimination half-life for iv data was 1.7 h, while the half-life for sc data increased from 1.3 h at 0.02 mg/kg to 80.7 h at 0.5 mg/kg dose. A possible reason could be a saturable absorption from the site of injection and ensuing flip-flop kinetics. Different doses of CET were administered in a constant volume which resulted in 0.02, 0.1, 0.25, and 0.5 mg/ml concentrations of CET in the injection formulation. Analysis of the mean concentration-time data following iv dosing and different sc doses by Loo-Riegelman's method (18) showed that the absorption was

complete at 8 h for 0.02 mg/kg dose, while for 0.5 mg/kg dose, about 5% of the dose remained unabsorbed even at the end of 5 days (Fig. 1). Hence, a population PK/PD model was developed to incorporate the dissolution rate limited absorption processes at each dose and to link the pharmacokinetics with the testosterone suppression.

Population PK/PD Modeling

Rats

The pharmacokinetics of CET in rats after iv data could be adequately described by a two-compartment model, represented by a set of differential equations:

$$\frac{dX_c}{dt} = -k_{12} \cdot X_c - (CL/V_c) \cdot X_c + k_{21} \cdot X_p$$

$$\frac{dX_p}{dt} = k_{12} \cdot X_c - k_{21} \cdot X_p$$

where, X_c and X_p are the amounts of CET in the central and the peripheral compartments, respectively; CL is the clearance and V_c is the volume of the central compartment, and k_{12} and k_{21} are the intercompartmental rate constants. For subcutaneous route, a pharmacokinetic model (Fig. 2) was developed to incorporate the saturable absorption processes. It was assumed that in the subcutaneous tissue after injection, a constant amount of CET (D_0) was present in the solution and the rest as a resoluble depot. Thus, the fraction of the dose present in the dissolved form (M, = D_0/dose) decreased at higher doses. Dissolution from the sc-depot into solution was saturable, and followed Michaelis-Menten kinetics. The model was represented by a set of differential equations:

$$\frac{dX_a}{dt} = -k_a \cdot M \cdot X_a + \frac{R_{\max} \cdot (1 - M) \cdot X_a}{R_m + (1 - M) \cdot X_a}$$

where $M = D_0/dose$

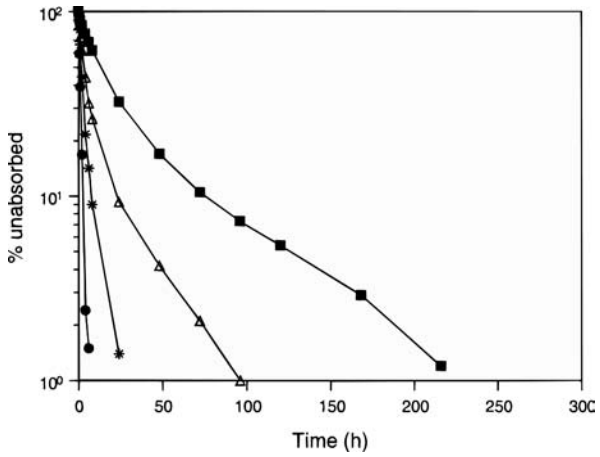

Fig. 1. Percent unabsorbed drug at different doses in rats, derived by Loo-Riegelman analysis (●: 0.02 mg/kg; *: 0.1 mg/kg; △: 0.25 mg/kg; ■: 0.5 mg/kg).

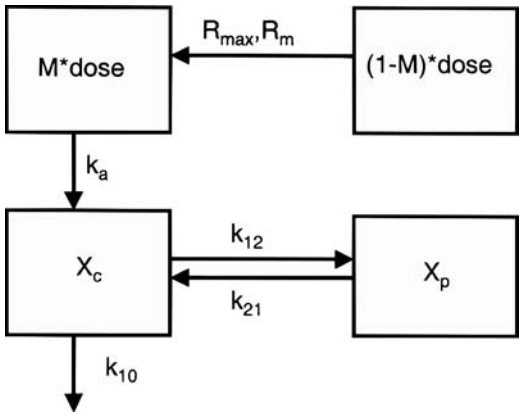

Fig. 2. Pharmacokinetic model for cetrorelix administered subcutaneously at different doses.

$$\frac{dX_c}{dt} = k_a \cdot M \cdot X_a - k_{12} \cdot X_c - (CL/V_c) \cdot X_c + k_{21} \cdot X_p$$

$$\frac{dX_p}{dt} = k_{12} \cdot X_c - k_{21} \cdot X_p$$

where, X_a is the amount of CET at the injection site, R_{max} is the maximum dissolution rate from the soluble depot at the injection site, and R_m is the amount of CET at which the dissolution rate is 50% of R_{max}.

Cetrorelix is an antagonist of LH-RH and suppresses the formation of testosterone. An indirect inhibitory sigmoid E_{max} model (19) was used to link the pharmacokinetics of CET to the PD effect of testosterone suppression:

$$\frac{dT}{dt} = T_0 \cdot k_e \cdot \left(1 - \frac{E_{\max} \cdot C^n}{IC_{50}^n + C^n}\right) - k_e \cdot T$$

where, C is the predicted CET concentration, T is testosterone concentration, T_0 is testosterone baseline, k_e is testosterone elimination rate constant, IC_{50} is CET concentration producing 50% of the maximum inhibitory effect, E_{max} = 1 (complete suppression) and n is the Hill factor, fixed at 2.

Dogs

Pharmacokinetics of CET after 0.1 mg/kg iv or sc administration could be best described by a three-compartment mammillary model. The inclusion of saturable absorption process was not necessary to fit the sc data. The PD model to explain the testosterone suppression in dogs was similar to that in rats, with the Hill factor fixed at 2.

A population model was developed for the PK/PD relationship in rats and dogs separately, by nonlinear mixed effect modeling, using P-PHARM software (Ver. 1.5, Innaphase, Champs sur Marne, France). The algorithm in P-PHARM is based on expectation-maximization method (20,21). The population approach examined the fixed (PK and PD model parameters) and the random (inter-subject variance of the PK and PD parameters, and residual variability) effects. The fixed effects were estimated using the simultaneous fitting of the PK- and PD models described above. The random effects were considered to consist of inter-subject variability (termed η with a standard deviation of ω for each PK or PD parameter), with the remaining

residual variability (termed as ϵ with a standard deviation of σ) within subjects. Maximum likelihood ratio, standard deviation for the fixed effects, Akaike Information Criterion, Residual error, and the distribution of the residuals were used as the indicators for the suitability of the population model to describe the PK/PD data.

Consistency Check of the PK/PD Estimates

To check the assumption on the error model and the distribution of random effect parameters, P-Pharm estimates the expected individual parameters (P_{exp}) given the population estimated values (using a Maximum a-Posteriori procedure) and the expected concentrations (C_{exp}) for each subject in the population and computes appropriate statistical tests to evaluate the distribution properties of the differences between the expected and observed data.

For each pharmacokinetic parameter, a value was estimated and the normalised Standardised Parameter Prediction Error (SPPE) values were computed as:

$$SPPE = \frac{P_{\exp} - P_{pop}}{SD(P_{pop})}$$

where P_{pop} is the population pharmacokinetic parameter and $SD(P_{pop})$ is the corresponding standard deviation.

For each concentration, a Standardised Concentration Prediction Error (SCPE) was calculated as follows:

$$SPPE = \frac{C_{obs} - C_{\exp}}{SD(C_{\exp})}$$

where C_{obs} represents the observed concentrations and $SD(C_{exp})$ represents the estimated standard deviation on the expected values computed using all sources of random variability including the residual error.

To assess the posterior distribution properties of the individual parameters and the residuals, the t-test was used to compare the mean of SPPE and SCPE to zero and the Kolmogorov-Smirnov test was used to compare the sampled distribution to the expected one (N (0,1)).

RESULTS

Assay Performance

Accuracy and precision of the CET radioimmunoassay (CET-RIA) and testosterone enzyme immunoassay (EIA) were determined by QC standards during application of the study samples. For the CET-RIA accuracy was 80–98.5% and precision was 11.4–25% with no differences between rat and dog plasma assays (12/9 batches). The testosterone EIA showed an accuracy of 83.1–121.7% and a precision of 3.4–12% and analogous to the RIA no differences between rat and dog plasma assays (10/7 batches).

Endogenous Testosterone Plasma Concentrations

In the control group of rats, no circadian rhythm of testosterone release was detected under study conditions. Testosterone concentrations in the control group were highly variable, as known from literature (22), ranging from 0.8–15.3 ng/ml with an overall mean of 4.5 ng/ml (n = 64) (Fig. 3). The means of the samples collected between 0–24 h were 4.1 ng/ml (n = 36) and from 24–264 h (same daytime) were 4.7 ng/ml (n = 32).

The testosterone plasma levels of male dogs are generally lower than in rats. In the literature, a range between 0.3 and 11 ng/ml with high intra-day fluctuation and a controversially discussed circadian rhythm is published (2,3,23). The pre-dose levels determined in this study were between 0.82 and 8.06 ng/ml, reflecting considerable variability in the normal range of baseline levels.

Studies in Rats

Rats were dosed with a single intravenous (0.1 mg/kg) or subcutaneous (0.02, 0.1, 0.25, and 0.5 mg/kg) injection of CET. The absolute bioavailability of CET in rats, based on AUC values at 0.1 mg/kg dose by iv and sc routes (Table II), was 100%. The extrapolated part of the AUCs after the last measured concentration was <2%. AUC's in the sc-route increased linearly with dose ($r^2 = 0.99$) demonstrating complete systemic availability between 0.02 and 0.5 mg/kg. The total body clearance (non-compartmental) was 5.4 ml/min/kg in the iv route and 4.7 ml/min/kg after sc-dosing, assuming complete absorption.

Population PK/PD-Modeling. The relationship between CET concentrations and testosterone suppression was modeled by population analysis. Following iv dose of CET the data was fitted to a biexponential equation. Following sc injection, the absorption process was dissolution rate limited and the dissolution of CET at the site of injection prior to systemic absorption followed Michaelis-Menten kinetics. The results are summarised in Table III. The microconstants K_{12}, K_{21}, and K_{10}, CL and Vc showed a log-normal distribution while other parameters showed normal distribution. The residual error was modeled as proportional to the square of the observation (weight = $1/y^2$). The population estimate of terminal half-life was 3.01 h and it was 3.03 ± 1.62 h for the iv dose. The PD model consisted of a sigmoid E_{max} model with the Hill factor (n) fixed at 2. The model without Hill factor (n = 1) did not adequately explain the hormone data after iv or sc administration of CET. The population estimates of IC_{50} and elimination half-life of testosterone were 1.39 ± 1.03 ng/ml and 1.68 h, respectively. The simulations of CET concentrations and the testosterone

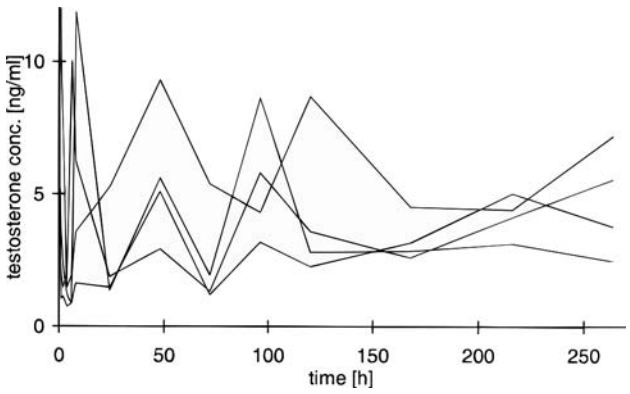

Fig. 3. Individual testosterone plasma levels following single injection of vehicle (5.2% mannitol) to male Wistar rats.

Table II. Mean (± SD) PK Parameters of CET and PD Parameters of Testosterone in Wistar Rats (n = 8; *n = 9) Following a Single Intravenous or Subcutaneous Injection at Different Doses of CET Acetate Salt and in Dogs (IV: n = 12, SC: n = 14) After 0.1 mg/kg Dose

	Rat					Dog	
	iv	sc				iv	sc
Parameter	0.1 mg/kg	0.02 mg/kg	0.1 mg/kg*	0.25 mg/kg	0.5 mg/kg	0.1 mg/kg	0.1 mg/kg
C_{max} ($ng \cdot ml^{-1}$)	291 ± 124	22.4 ± 3.3	53.3 ± 11.1	68.6 ± 9.0	53.6 ± 14.8	679 ± 73	197 ± 54
t_{max} (h)	—	1.2 ± 0.5	1.8 ± 0.4	1.3 ± 0.5	1.3 ± 0.5	—	1.6 ± 0.8
$AUC_{0-\infty}$ ($ng \cdot h \cdot ml^{-1}$)	349 ± 135	75 ± 9	363 ± 66	619 ± 78	1180 ± 232	1551 ± 304	1507 ± 18.6
MRT (h)	1.9 ± 1.5	2.5 ± 0.3	6.6 ± 1.6	15.0 ± 3.9	37.8 ± 6.1	5.7 ± 0.5	8.3 ± 1.7

suppression at each CET dose, using the mean post-hoc estimates of the parameters for each dose group, are represented in Figure 4. A good relationship was observed between the observed data (both PK and PD) and the data predicted based on the given PK/PD and the distribution models ($Y_{pred} = 1.03 * Y_{obs} + 0.87$ ($r = 0.91$; d.f. = 398; $p < 0.001$)). The distribution models for the inter-individual variability of the parameters and the residual error were validated by SPPE and SCPE tests executed by P-PHARM software, which showed that the sampled distribution was not statistically different from the expected one (N (0, 1)).

Studies in Dogs

The average pharmacokinetic parameters in dogs are listed in Table II. Mean C_{max} of 197 ng/ml after sc injection was observed at 1.6 h. The absolute bioavailability of CET by sc route, based on the AUC values (Table II) was 97%. The extrapolated part of the AUCs of the individuals was <3%. The total body clearance, assuming complete absorption, was 1.1 ml/min/kg in both routes.

Testosterone suppression was linked to the CET concentrations by a sigmoid E_{max} model, similar to rats. After 1–2 hours post-dosing, the testosterone levels in all dogs declined reaching levels below 0.6 ng/ml after 4 h. All individuals of both injection routes returned to testosterone baseline levels within 48 h.

The population pharmacokinetics of CET and its relationship with the testosterone suppression in dogs were explained by a three-compartment model with a terminal elimination half-life of 9.3 h (Table III). The Hill factor in the PD equation was fixed at 2. The model parameters showed a normal distribution, with a heteroscedastic model, proportional to the square of observation (weight = $1/Y^2$). The analysis of SPPE and SCPE showed that the distribution was not significantly different from the expected one (N (0, 1)). There was a significant correlation between the observed and predicted PK and PD data ($Y_{pred} = 1.00 * Y_{obs} + 2.44$ ($r = 0.99$; d.f. = 382; $p < 0.001$)).

The PK/PD relationships after iv and sc doses of 0.1 mg/kg doses in dogs are represented in Fig. 5. The elimination rate constant (k_e) of testosterone was 0.65 h^{-1} equivalent to a half-life of 1.1 h.

Table III. Population Pharmacokinetic and Pharmacodynamic Parameters for Cetrorelix and Testosterone Suppression in Rats and Dogs

Parameter	Rat	Dog
V_c ($l \cdot kg^{-1}$)	0.328 (28%)	0.101 (13%)
V_{ss} ($l \cdot kg^{-1}$)	0.600 (43%)	0.455 (19%)
CL ($l \cdot h^{-1} \cdot kg^{-1}$)	0.433 (33%)	0.067 (9.9%)
k_{12} (h^{-1})	0.307 (43%)	2.51 (16%)
k_{21} (h^{-1})	0.310 (51%)	1.19 (21%)
k_{13} (h^{-1})	—	0.096 (38%)
k_{31} (h^{-1})	—	0.074 (24%)
R_{max}($\mu g \cdot h^{-1} \cdot kg^{-1}$)	4.32 (36%)	—
R_m ($\mu g \cdot kg^{-1}$)	29.8 (11%)	—
D_0 ($\mu g \cdot kg^{-1}$)	30.4 (12%)	—
k_a (h^{-1})	1.05 (29%)	0.699 (24%)
IC_{50} ($ng \cdot ml^{-1}$)	1.39 (74%)	1.24 (24%)
T_0 ($ng \cdot ml^{-1}$)	9.75 (29%)	5.56 (26.6%)
k_e (h^{-1})	0.412 (15%)	0.654 (18%)

DISCUSSION

Cetrorelix is completely bioavailable after subcutaneous injection in rats and dogs at a dose of 0.1 mg/kg. AUCs after sc dosing of 0.02–0.5 mg/kg in rats increased proportional to dose showing complete extent of absorption over the investigated dose range. C_{max} did not increase in doses higher than 0.1 mg/kg and the elimination half-lives calculated from the terminal portions of the curves increased from 1.3 h at 0.02 mg/kg to 80 h at 0.5 mg/kg, indicating flip-flop kinetics. It was assumed that in the subcutaneous tissue after injection CET is present as a resoluble depot. The dissolution of CET at the site of injection prior to systemic absorption followed Michaelis-Menten kinetics and was the rate-limiting step.

The suppression of endogenous testosterone production in the gonads by CET is based on the competitive binding to the LH receptors in the pituitary gland, inhibiting the stimulatory action of LH-RH on the production of LH and FSH. The duration of suppression is dependent on the CET concentration in this organ. After systemic exposure, CET plasma levels are a reliable correlate for CET concentration in the pituitary. EC_{50} estimates in both the species were 1–2 ng/ml and were independent of dose and prior duration of suppression, confirming the competitive character of the mode of action.

A down-regulation model for the stimulatory effect of chorionic gonadotropin on the testosterone production in normal men has been proposed (24). It was proposed that chorionic gonadotropin can bind to two sites present on the LH receptor and the effect is proportional to one-site bound concentration. Binding to the second site at high gonadotropin concentration causes the down-regulation of the receptors. Down-regulation of LH-RH receptors has been observed after long-term administration of cetrorelix in rats (25). Interdependency in the suppression of LH and testosterone after the administration of another

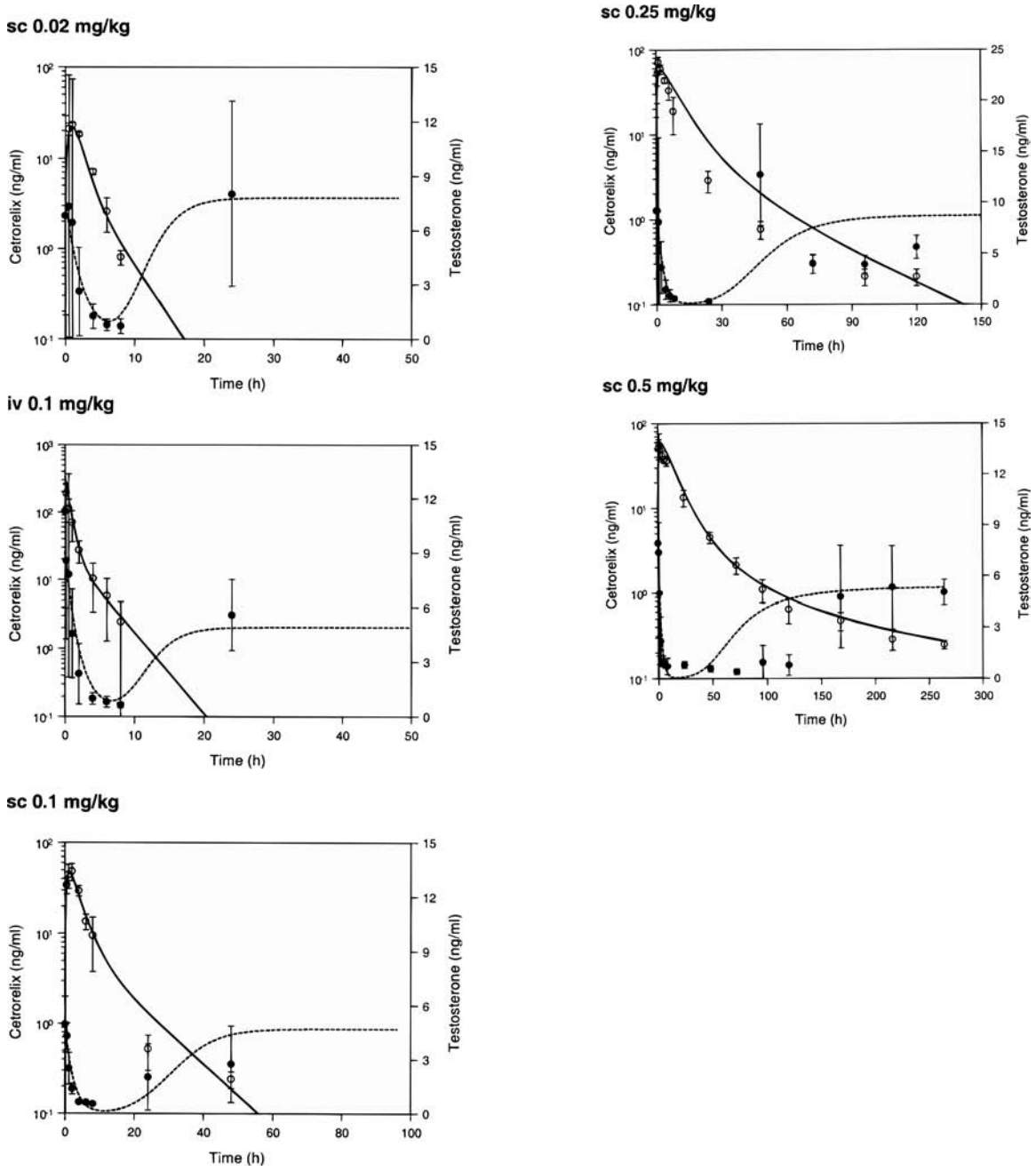

Fig. 4. PK/PD relationship between cetrorelix concentrations and testosterone suppression in rats (○: PK, ●: PD).

LH-RH antagonist antide to healthy men has been modeled by a bivariate control system wherein, the LH effect on testosterone depends on previous LH exposure and that LH depends on previous testosterone exposure, resulting in the LH overshoot after the antide induced suppression (26).

A population PK/PD model was developed to explain the pharmacokinetics of CET in rats that involved dissolution rate-limited absorption kinetics and to link the CET concentrations to the testosterone suppression. The pharmacokinetics of CET in dogs was explained by a three-compartment model. A Hill factor of 2 was necessary to explain the hormonal suppression in both rats and dogs at all doses. The goodness of the fits in the current study indicates that the data can be described sufficiently well without consideration of the circadian rhythm.

The population estimates for IC_{50} were 1.39 ± 1.03 ng/ml in rats and 1.24 ± 0.30 ng/ml in dogs. The elimination half-life of testosterone was calculated to be about 1.7 h in rats and 1.1 h in dogs. In both species, no difference in the onset of hormonal suppression between the iv and the sc routes was detectable indicating that the time necessary to reach sufficient CET levels in pituitary gland is negligible in comparison with the elimination half-life of testosterone.

A comparison of the elimination half life of CET with publications for LH-RH and other LH-RH analogues in three

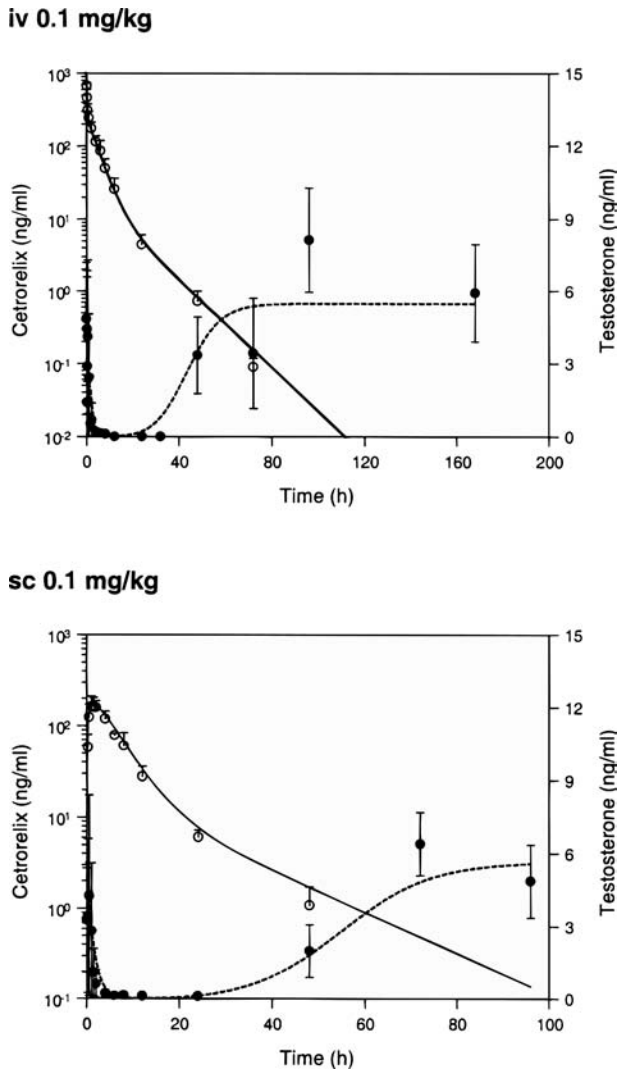

Fig. 5. PK/PD relationship between cetrorelix concentrations and testosterone suppression in dogs (○: PK, ●: PD).

species (Table I) shows a consistent 3 to 4 fold increase of the elimination half lives from LH-RH to LH-RH agonists and a further increase of the same magnitude from LH-RH agonists to LH-RH antagonists. The increasing stability against proteolytic enzymes dependent on the number of D-aminoacids introduced surely contributes to the described effect (27,28). CL after iv dosing in rats and dogs compared with human CL (Pechstein *et al.*, submitted) was found to be a linear function of body weight ($r^2 = 0.999$).

The time dependency between single peaks of LH-RH, LH and the testosterone response in rats (29) and dogs (3,30) has been studied extensively. The corresponding LH maximum was detected within 5 min after LH-RH dosing in dogs, whereas the testosterone response was delayed for 10 min and the maximum was observed 40 min to 1 h after dosing. In this study, both testosterone production and elimination were characterised. It has been reported that male dogs produce 4.5 testosterone pulses per 6 h (23). This endogenous rhythm is the result of testosterone elimination and production caused by LH-RH feedback.

CONCLUSIONS

CET is completely bioavailable after subcutaneous injection. The rate of absorption at dose levels above 0.1 mg/kg is limited as concluded from constancy of C_{max}. Complete systemic availability, however, can be concluded for the investigated dose range from the proportionality of AUCs with doses up to 0.5 mg/kg. A pharmacokinetic model with dissolution rate limited absorption process was required to explain the dose-independent peak plasma concentrations in rats and the apparent increase in the terminal half-life. No sex difference could be observed at any of the dose levels. Population PK/PD models were developed separately in rats and dogs. The population model could effectively link the drug levels with the hormonal suppression at all doses. PK/PD modeling with CET and testosterone plasma concentrations in both male rats and dogs led to a good correlation using the sigmoid E_{max} model.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Mrs. S. Pawlik, J. Kulle, P. Lapp, M. Meckel, G. Motzheim and A. Nonnenmacher for their excellent technical assistance.

REFERENCES

1. E. Knobil. The neuroendocrine control of the menstrual cycle. *Recent Prog. Horm. Res.*, **36**:53–80 (1980).
2. S. Fukuda. Circadian rhythm of serum testosterone levels in male beagle dogs—effects of lightning time zone. *Exp. Anim.*, **39**(1):65–68 (1990).
3. L. R. DePalatis, J. Moore, and R. E. Falvo. Plasma concentrations of testosterone and LH in the male dog. *J. Reprod. Fert.*, **52**: 201–207 (1978).
4. S. Bajusz, M. Kovacs, M. Gazdag, L. Bokser, T. Karashima, V. J. Csernus, T. Janaky, J. Guoth, and A. V. Schally. Highly potent antagonists of luteinizing hormone-releasing hormone free of edematogenic effects. *Proc. Natl. Acad. Sci. USA* **85**:1637–1641 (1988).
5. M. Schwahn, P. Romeis, G. Peter, H. Derendorf, and M. Herbst. Absolute bioavailability and dose proportionality of CET, a novel LH-RH antagonist, in male and female rats. *Arch. Pharmacol.* **355** (4):R8 (1997).
6. H. Berger, N. Heinrich, H. Schäfer, I. Baeger, and B. Mehlis. Gonadotropin-releasing hormone (GnRH) pharmacokinetics: peptide hormone pharmacokinetics needs clarification. *Life Sci.* **42**:985–991 (1988).
7. N. I. Chu, R. L. Chan, K. M. Hama, and M. D. Chaplin. Disposition of Narfarelin acetate, a potent luteinizing hormone-releasing hormone agonist, in rats and rhesus monkeys. *Drug Metab. Dispos.* **13**:560–565 (1985).
8. H. Berger, N. Heinrich, E. Albrecht, U. Kertscher, J. Oehlke, M. Bienert, H. Schäfer, I. Baeger, and B. Mehlis. Gonadotropin-releasing hormone (GnRH) analogs: relationship between their structure, proteolytic inactivation and pharmacokinetics in rats. *Regul. Pept.* **33**:299–311 (1991).
9. E. Ezan, K. Drieu, M. Chapelat, C. Rougeot, and F. Dray. Radioimmunoassay of [D-Trp^6]-luteinizing hormone releasing hormone: its application to animal pharmacokinetic studies after single injection and long-acting formulation administration. *Regul. Pept.* **14**:155–167 (1986).
10. H. Okada, Y. Inoue, T. Heya, H. Ueno, Y. Ogawa, and H. Touchi. Pharmacokinetics of once-a-month injectable microspheres of leuprolide acetate. *Pharm. Res.* **8**:787–791 (1991).
11. E. Hudon, J. J. Rondeau, V. Lenaerts, P. Wüthrich, F. Boutignon, R. Deghenghi, S. Marleau, N. Yamagutchi, A. Adam, and H. Ong. Radioimmunoassay of meterelin and pharmacokinetics after single injection and implant administration in dogs. *J. Pharm.*

Sci. **86**:172–178 (1997).
12. F. Haviv, E. N. Bush, J. Knittle, and J. Greer. LHRH Antagonists. In Borchard (eds), *Integration of pharmaceutical discovery and development*, Plenum Press, New York, 1998.
13. S. Sorensen, J. J. Rondeau, V. Lenaerts, F. Boutignon, P. Wüthrich, R. Deghenghi, A. Adam, and H. Ong. Radioimmunoassay of antarelix, a luteinising hormone releasing-hormone antagonist, in plasma and its application for pharmacokinetic study in dogs. *J. Immunoassay* **17**:205–226 (1996).
14. R. L. Chan, W. Ho, A. S. Webb, J. A. LaFargue, and C. A. Nerenberg. Disposition of detirelix, a potent luteinising hormone-releasing hormone antagonist, in rats and monkeys. *Pharm. Res.* **5**:335–340 (1988).
15. R. L. Chan, S. C. Hsieh, P. E. Haroldsen, W. Ho, and J. J. Nestor, Jr. Disposition of RS-26306, a potent luteinizing hormone-releasing hormone antagonist, in monkeys and rats after single intravenous and subcutaneous administration. *Drug Metab. Dispos.* **19**:858–864 (1991).
16. V. J. Csernus, B. Szende, K. Groot, T. W. Redding, and A. V. Schally. Development of radioimmunoassay for a potent luteinizing hormone-releasing hormone antagonist. *Drug Res.* **40**: 111–118 (1990).
17. H. H. Raffel, M. Locher, H. O. Borbe. High-performance liquid chromatographic assay for the determination of the decapeptide cetrorelix, a novel luteinizing hormone-releasing hormone antagonist, in human plasma. *J Chromatography B.* **653**:102–105 (1994).
18. M. Gibaldi and D. Perrier. *Pharmacokinetics*. 2nd Ed. Marcel Dekker Inc., 1982, pp. 155–161.
19. N. L. Dayneka, V. Garg, and W. J. Jusko. Comparison of four basic models of indirect pharmacodynamic responses. *J. Pharmacokin Biopharm.* **21**:457–478 (1993).
20. F. Mentre and R. Gomeni. A two step iterative algorithm for estimation in nonlinear mixed-effect models with an evaluation in population pharmacokinetics. *J. Biopharm. Stat.* **5**:141–158 (1995).
21. User Manual, P-Pharm, (Ver. 1.5), Innaphase, Champs sur Marne, France (1994).
22. A. Bartke, R. E. Steele, N. Musto, and B. V. Caldwell. Fluctuations in plasma testosterone levels in adult male rats and mice. *Endocrinol.* **92**:1223–1228 (1973).
23. A. R. Günzel-Apel, H. G. Brinckmann, and H. O. Hoppen. Dynamics of LH and testosterone secretion in male beagles of different age. *Reprod. Dom. Anim.* **25**:78–86 (1990).
24. J-M. Gries, A. Munafo, H. C. Porchet, and D. Verotta. Down-regulation models and modeling of testosterone production induced by recombinant human choriogonadotropin. *J. Pharmacol. Exp. Ther.* **289**:371–377 (1999).
25. G. Halmos, A. V. Schally, J. Pinski, M. Vadillo-Buenfil, and K. Groot. Down-regulation of pituitary receptors for luteinizing hormone-releasing hormone (LH-RH) in rats by LH-RH antagonist Cetrorelix. *Proc. Natl. Acad. Sci. USA* **93**:2398–2402 (1996).
26. K. E. Fattinger, D. Verotta, H. C. Porchet, A. Munafo, J-Y. Le Cotonnec, and L. B. Sheiner. Modeling a bivariate control system: LH and testosterone response to the GnRH antagonist antide. *Am. J. Physiol.* **271**:E775–E787 (1996).
27. A. Lasdun, S. Reznik, C. J. Molineaux, and M. Orlowski. Inhibition of endopeptidase 24.15 slows the in vivo degradation of luteinizing hormone- releasing hormone. *J. Pharmacol. Exp. Ther.* **251**:439–447 (1989).
28. B. Horsthemke, H. Knisatschek, J. Rivier, J. Sandow, and K. Bauer. Degradation of luteinizing hormone-releasing hormone and analogs by adeno-hypophyseal peptidases. *Biochem. Biophys. Res. Commun.* **100**:753–759 (1981).
29. K. Hakola, D. Pierroz, A. Aebi, B. A. M. Vuagnat, M. L. Aubert, and I. Huhtaniemi. Dose and time relationship of intravenously injected rat recombinant luteinizing hormone and testicular testosterone secretion in the male rat. *Biol. Reproduction* **59**:338–343 (1998).
30. B. W. Knol, S. J. Dielemann, M. M. Bevers, and W. E. van den Brom. GnRH in the male dog: dose-response relationship with LH and testosterone. *J. Reprod. Fert.* **98**:159–161 (1993).