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The dosing solution influence on the pharmacokinetics of degarelix, a new GnRH0 antagonist, after s.c. administration to beagle dogs

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Abstract

Objective: Degarelix (FE200486) is a new GnRH-receptor antagonist intended for the treatment of prostate cancer. The objective of the present analysis was to evaluate the pharmacokinetics of degarelix after subcutaneous (s.c.) and intra-muscular (i.m.) administration to male beagle dogs, and to determine the influence of the different dosing conditions on the absorption profile of degarelix. *Methods*: Degarelix was administered to 27 dogs and plasma concentrations were measured. The dosing conditions varied with respect to route (s.c. or i.m.), dose (0.25–1.5 mg/kg), solution strength (1.25–40 mg/ml) and volume administered (0.15–2.9 ml). Data were analysed by use of non-linear mixed effect modelling to characterize the pharmacokinetics, in particular the relationship between dosing conditions and rate, and extent of absorption. *Results*: After s.c. and i.m. administration of degarelix, the plasma concentration versus time profile was best described by applying a two-compartment model, with two input functions: a fast first-order input function to describe the rapid initial increase in the plasma concentration levels, and a slow first-order input function to describe the prolonged absorption profile of degarelix. Intra-muscular as opposed to s.c. administration led to a more rapid absorption of degarelix, reaching a mean maximum concentration of 64 and 31 ng/ml roughly 2.0 and 3.7 h after administration, respectively. The slow absorption half-life was found to be 268 h (∼11 days). The relative fraction absorbed was found to vary with the concentration of the dosing solution. The present analysis suggested that the absorbed fraction was reduced by approximately 50% when the concentration in dosing solution was increased from 1.25 to 40 mg/ml. The rate of the initial absorption component was also dependent on the concentration in the dosing solution, with slower absorption at higher concentrations. *Conclusion*: Through varying the dosing conditions and by applying a joint analysis of all data, the important factors determining the complex absorption of degarelix could be described.

Keywords: FE200486; Degarelix; NONMEM; Non-linear mixed effects; Prostate cancer; Pharmacokinetics

1. Introduction

Prostate cancer has become the most common cancer and is second only to lung cancer as a cause of male cancer-related deaths. Prostate cancer has for many years been treated with GnRH-receptor agonists. An agonist initially stimulates the hormone secretion, but will subsequently cause a down-regulation of GnRH-receptors resulting in the inhibition of luteinizing hormone production upon continuous exposure, which in turn causes a suppression of testosterone and dihydrotestosterone, on which continued growth of prostate cancer cells depends (Cook and Sheridan, 2000). In such clinical situations where an immediate and profound suppression of gonadotrophins is desired, the use of GnRH antagonists that cause an immediate and dose-related inhibition of LH and FSH by competitive blockade of the GnRH-receptors is more advantageous (Reissmann et al., 2000). Several experiments have demonstrated the effect of GnRH antagonists, e.g. the response of cetrorelix given every 12 h s.c. to prostate cancer patients was obvious: a significant decrease in bone pain, relief in urinary outflow obstruction, and reversal of

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the signs of prostatism (Gonzalez-Barcena et al., 1994). What has limited the use of the marketed GnRH antagonists in the treatment of prostate cancer so far has been their short duration of action, as compared to the agonists where at least 1 month depot formulations are available (Kuhn et al., 1997; Giberti et al., 1988). In order to prolong the duration of action of the antagonist, several studies have been performed with sustained release formulations (microcapsules or microgranules) (Korkut et al., 1991; Redding et al., 1992). Degarelix is a competitive, selective GnRH receptor antagonist that is being developed for the treatment of prostate cancer. In animal studies, degarelix has been demonstrated to effectively lower both the plasma testosterone levels and the growth rate of tumours to levels comparable to those obtained after surgical castration (Broqua et al., 2002; de Pinieux et al., 2001). Subcutaneous injection of degarelix will result in the formation of a depot, from which degarelix is slowly released into the circulation (Broqua et al., 2002). The objective of the present non-linear mixed effects analysis was to clarify which covariates control the release of degarelix from the depot, and to assess the impact of these covariates on the pharmacokinetic profile of degarelix in order to optimize the dosing regimen.

2. Materials and methods

2.1. Experimental design

Degarelix (FE200486, Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph (L-hydroorotyl)-D-4Aph (carbamoyl)-Leu-ILys-Pro-D-Ala-NH2) is a linear decapeptide amide containing seven unnatural amino acids (Jiang et al., 2001). It is a long-acting, competitive GnRH antagonist with high affinity and selectivity for GnRH receptors with high water solubility and low histamine-releasing properties (Jiang et al., 2001). The pharmacokinetic analysis described in the present report comprises two different studies in beagle dogs. Briefly, 27 dogs were dosed with degarelix at four different dose levels (Table 1). The concentration levels in the dosing solution ranged from 1.25 to 40 mg/ml. The following covariates were available for testing: the dose administered, the concentration of degarelix in the dosing solution (mg/ml), the amount administered (ml/dog), i.m. versus s.c. administration, and the body weight of the dogs (kg). Plasma samples were collected predose, 0.5, 1, 2, 3, 4, 24, 144, 312, 504, 648 and 984 h (41 days) after dosing in the first study, and predose, 0.5, 1, 2, 3, 4, 24, 144, 312, 648, 984, 1320, 1656, 1992, 2160, 2832 and 3504 h (56 days) after dosing in the second study. The plasma samples were analysed for degarelix using two different assays. A validated liquid chromatography-tandem mass spectrometric (LC-MS/MS) method was used for analysis of all samples from experiments with the 5 mg/ml dosing solution, whereas the remaining samples were assayed by a validated radioimmunoassay (RIA). The LC-MS/MS method is based on solid phase extraction (using cation exchanger) followed by reversed phase liquid chromatography and tandem mass spectrometry. The RIA method is a competitive immunoassay based on polyclonal antibodies and monoiodinated tracer. Cross-assay validation has demonstrated comparable results between the two assays. For the radioimmunoassay, the lower limit of quantification (LLOQ) was 0.5 ng degarelix/ml dog plasma. The intra- and inter-assay precision (expressed as % coefficient of variation) was less than or equal to 8.9% or 15%, respectively. The accuracy was less than or equal to ±6%. For the LC-MS/MS assay the lower limit of quantification (LLOQ) was 0.5 ng FE 200486/ml dog plasma. The intra- and inter-assay precision was less than or equal to be 13% or 11%, respectively. The accuracy was less than or equal to ±3.2%.

Table 1
Experimental design. Degarelix was administered to 27 beagle dogs, three dogs per group, at four different dose levels

Dose level (mg/kg)	Route of administration	Conc. in dose solution (mg/ml)	Volume given per dog (ml)
0.25	s.c.	1.25	2–2.4
0.5	s.c.	2.5	1.9–2.5
	s.c.	5	0.7–0.9
	i.m.	5	0.7–1
1.0	s.c.	5	1.5–1.9
	s.c.	10	0.6–0.9
	s.c.	20	0.3–0.4
	s.c.	40	0.15–0.2
1.5	s.c.	5	2.3–2.9

2.2. Analysis of data

2.2.1. Modelling approach

The pharmacokinetic parameters were assessed by non-linear mixed effect modelling through the NONMEM program, version V (Beal and Sheiner, 2000). In the model-building process, the first-order (FO) estimation method was used, whereas only the final model selected was run by use of the conditional first-order method with interaction (FOCE). Several different models with different number of compartments and input functions were tested (Table 2). The inter-individual variability was modelled by an exponential error model. Different intra-individual error models were tested: an additional error model, a proportional error model, and a combination error model. The goodness of fit was evaluated by graphic analysis of predicted versus observed concentrations (distribution of the points around the unity line), by weighted residuals versus predicted concentrations, and by comparing the objective function values using Xpose 3.0 (Jonsson and Karlsson, 1999).

Table 2
List of the different covariate relationships included into the model. At each step, the relevant parameters were tested against the different covariates available

Covariates	Method	No. of parameters	OFV
No covariates included	FO	13	943.639
Sigmoidal E_{max} model included on F_{rel}	FO	15	893.234
k_{fast} vs. ADM (i.m./s.c.)[a]	FO	16	875.283
Sigmoidal E_{max} model on k_{fast} after s.c. administration[b]	FO	18	832.923
As above	FOCE	18	830.793

[a] Note that two different relationships are included in the k_{fast}. First k_{fast} was allowed to vary with the administration form (one k_{fast} for i.m. and one k_{fast} for s.c.).
[b] Thereafter a sigmoidal relationship was included in the k_{fast} obtained for the s.c. administration.

2.2.2. Covariates

In the first step, individual estimates of the pharmacokinetic parameters were obtained as empirical Bayes estimates based on a NONMEM fit using no covariates. In the second step, the individual pharmacokinetic parameter estimates were regressed on the covariates using a variety of models (linear, log-linear and sigmoidal relationships). The covariate resulting in the largest improvement in the population model was included in the model. After inclusion of the first covariate, the procedure mentioned above was repeated. The new set of individual random effect parameters was plotted against the available covariates, and their relationship evaluated by use of non-linear regression methods; then the covariates resulting in the largest improvement of the population model were included in the model, and so forth.

3. Results

The following pharmacokinetic models were tested: a one-compartment model with first and zero-order input, a one-compartment model with two first-order inputs, a two-compartment model with first- and zero-order input, and a two-compartment model with two first-order input functions. The pharmacokinetic observations were best

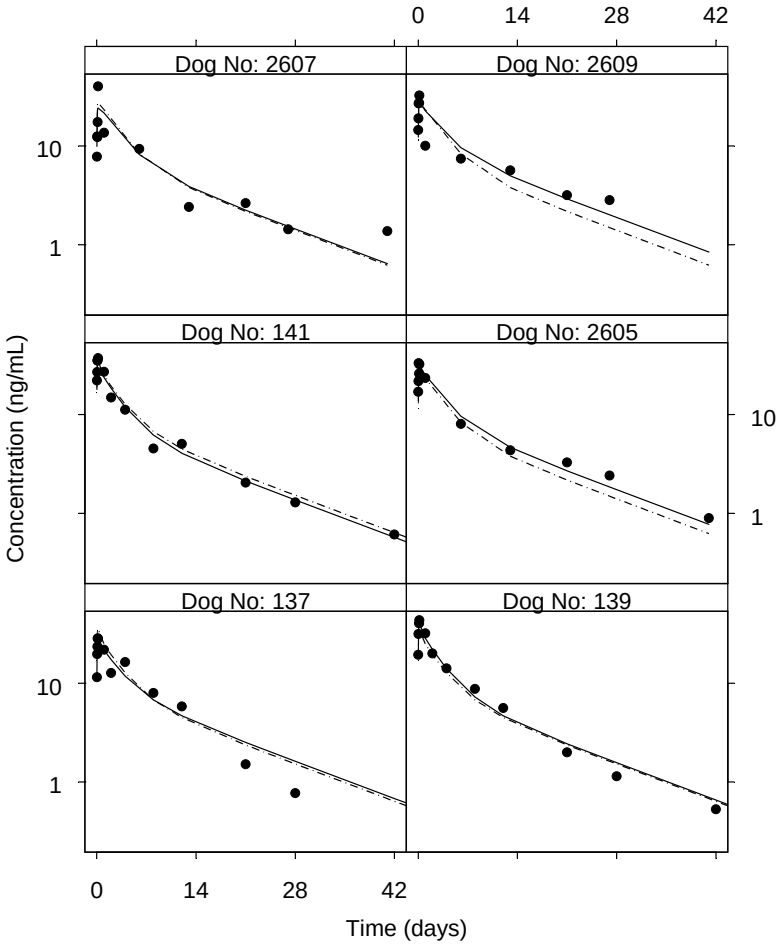

Fig. 1. Individual plots of the plasma concentration vs. time profile after subcutaneous administration of 0.5 mg degarelix/kg to male beagle dogs. The dots indicate the plasma concentration levels observed, the solid line indicates the concentrations predicted by the individual population model, and the dotted line the concentrations predicted by the population model.

described by a two-compartmental model with two different input functions: a fast first-order input describing the initial rapid increase in the plasma concentration levels, and a slow first-order input to account for the prolonged release from the depot formulation (Fig. 1). The parameters of the model include: the two first-order absorption rate constants (k_{fast} and k_{slow}), the fraction of the dose given by the fast input function (FR), the fraction of the dose given by the slow input function (1-FR), the clearance (CL/F), the inter-compartmental clearance (Q/F), the volumes of the central (V_c/F) and peripheral (V_p/F) compartments, as well as the relative bioavailability (F_{rel}). F_{rel} was set at one for the lowest dosing solution concentration, but it was allowed to vary for the other concentration levels. If the bioavailability was independent of the concentration in the dosing solution, F_{rel} was expected to be one for all dose concentration levels tested. It should be noted that the F used in e.g. CL/F represents a correction for the actual bioavailability and has nothing to do with the F_{rel} parameter. The intra-individual variation was best described by a combined additive and proportional error model.

In order to elucidate the influence of the dose formulation tested in the present experiment, the different formulation characteristics were included in the modelling as covariates (Table 2). The relative bioavailability fraction (F_{rel}) was found to be dependent on the concentration in the dosing solution, where an increase in the dose concentration led to a decrease in the absorbed fraction (Fig. 2). Inclusion of the dose concentration as a covariate led to a significant decrease in the objective function value (Table 2). The relationship between the F_{rel} and the concentration in the dosing solution was best described by the following equation:

$$F_{rel} = F_{rel,0}\left(1 - \frac{E_{MAX}\,CDS^{\gamma}}{EC_{50}^{\gamma} + CDS^{\gamma}}\right)$$

where $F_{rel,0}$ represents the baseline estimate of the relative bioavailability (F_{rel}) and its value was fixed at one; CDS is the concentration in the dosing solution; EC_{50} represents the concentration in the dosing solution at which F_{rel} obtains half the maximum reduction in the bioavailability; E_{MAX} represents the maximum reduction in the F_{rel}, and the sigmoidicity factor is represented as γ.

The route of administration (s.c. versus i.m.) was included in the fast first-order input rate constant (k_{fast}) as a covariate relationship, which also reduced the objective function value significantly (Table 2). The fast first-order rate constant did not vary only with the route of administration, but also with the concentration in the dosing solution. Lower dosing solution concentrations led to a more rapid first-order input

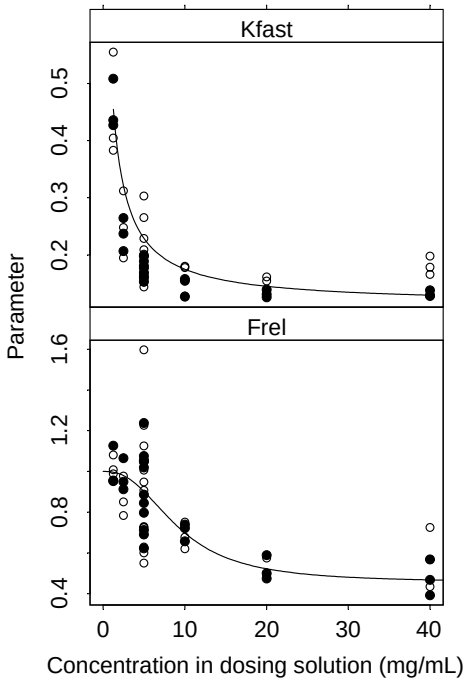

Fig. 2. Depiction parameter-covariate relationships included in the modelling of degarelix after s.c. administration to beagle dogs. The filled circles indicate the individual parameters estimated by the final model and the solid line indicates the typical relationship. The open circles indicate the estimated individual parameters prior to including the covariates in the model.

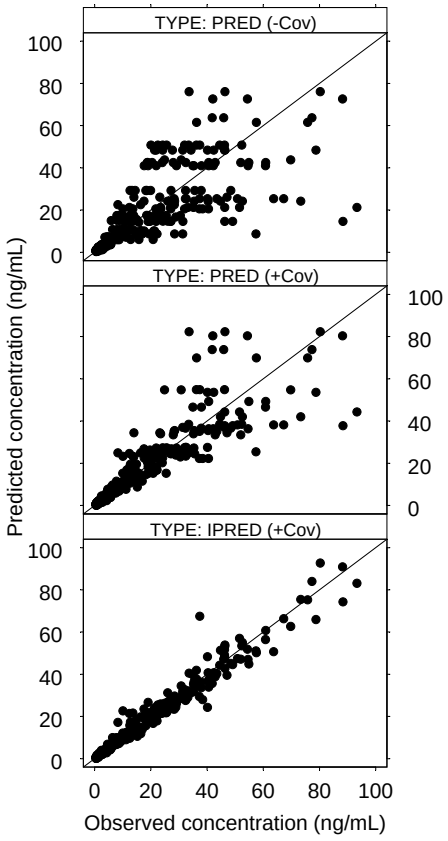

Fig. 3. Predicted vs. observed plasma concentrations after subcutaneous administration of degarelix to beagle dogs (dose range: 0.5–1.5 mg/kg). Top graph: the population model before including the covariate relationships. Middle graph: final population model graph. Lower graph: observed values plotted against individually predicted ones for the final model.

Table 3
Pharmacokinetic results obtained after fitting a two-compartment model with two first-order input functions to degarelix plasma concentration data from beagle dogs

Parameter	Unit of measure	Final model	
		Estimate (R.S.E.%)	CV% (R.S.E.%)
CL/F	l/(h kg)	0.0982 (4.4)	–
V_c/F	l/kg	1.20 (30)	–
V_p/F	l/kg	5.21 (32)	–
Q/F	l/(h kg)	0.961 (23)	–
$k_{fast(i.m.)}$	h^{-1}	0.413 (26)	15 (51)
$k_{fast,0}$	h^{-1}	0.455 (16)	
$k_{fast(EC50)}$	mg/ml	1.92 (16)	–
$k_{fast(Emax)}$	h^{-1}	0.129 (19)	–
k_{slow}	h^{-1}	0.00259 (14)	–
$F_{rel,0}$	NA	1	
$F_{rel(Emax)}$	NA	0.546 (20)	
$F_{rel(EC50)}$	ng/ml	9.17 (17)	17 (30)
$F_{rel(\gamma)}$	NA	2.50 (54)	
FR	NA	0.446 (19)	7 (34)
Experimental error	%	17.8 (11)	–
Add error	ng/ml	0.320 (16)	–

k_{fast}: First-order absorption rate constant describing the fast absorption phase; k_{slow}: first-order absorption rate constant describing the slow absorption phase; FR: fraction describing the part of the dose being absorbed slowly (e.g. a Fraction of 0.7 would indicate that 70% of the dose has been absorbed in the fast phase controled by k_{fast}); $F_{rel,0}$: The relative bioavailability at baseline (fixed at one). R.S.E.: the relative standard error of the estimate.

after s.c. administration. This relationship was implemented in the model by the following equation:

$$k_{fast} = k_{fast,0}\left(1 - \frac{E_{MAX}\,CDS}{EC_{50} + CDS}\right)$$

However, in order to avoid extrapolation beyond the data range, the model was re-parameterised so that $k_{fast,0}$ represents the estimate of k_{fast} at a CDS of 1.25 mg/ml; E_{MAX} represents the estimate of k_{fast} at a CDS of 40 mg/ml; CDS is the concentration in the dosing solution and EC_{50} represents the concentration in the dosing solution at which the k_{fast} obtains half the maximum reduction. The relationship between the concentration in the dosing solution and k_{fast} implies that low concentration levels in the dosing solution will lead to high values for k_{fast} (Fig. 2). Inclusion of the covariate relationships significantly improved the population model by reducing the scattering around the unity line (Fig. 3) as well as the inter-individual variability in the population, while the intra-individual variability remained almost constant within the population (Table 3). In the final model, ETA values were included in the following parameters: in those controlling the fast input (k_{fast}), in those controlling the relative bioavailability (k_{fast}), and finally in the FR parameter controlling the fraction being absorbed via the fast and the slow route. The final model was rerun using the FOCE method with interaction, which resulted only in marginal changes in the parameter estimates.

4. Discussion

Degarelix is a new potent GnRH-receptor antagonist. It has previously been suggested that an in-situ depot formation is most likely responsible for the prolonged efficacy of degarelix (Broqua et al., 2002). The nature of the depot has not been well established as yet, but it appears to have a gel structure. It is speculated that the gel is formed as soon as degarelix comes into contact with, e.g. tissue proteins after s.c. administration, resulting in a slow release of the active compound from the depot. This special nature of the depot that is formed after s.c. or i.m. administration of degarelix is an advantage in the treatment of prostate cancer, because active plasma concentration levels can be maintained for a prolonged period of time (Broqua et al., 2002). Several different factors may affect the formation and the viscosity of the gel and hence the release profile of degarelix. The complexity of this gel structure provides us with a tool for controlling the release pattern of degarelix from the depot, but in order to control the release, an understanding of the release profile is crucial.

In the present analysis, the main objective of using a non-linear mixed effect approach was to investigate the nature of the absorption kinetics of degarelix after s.c. and i.m. administration to beagle dogs, and to examine which covariates influenced the absorption process. An advantage of using non-linear mixed effects modelling is the possibility it offers to describe the variability in the population. The application of NONMEM enables us to estimate the mean parameter values of the population, and variances and covariances simultaneously for all individuals. Another valuable aspect of the mixed effects approach as opposed to the standard two-stage approach is the possibility of including covariates directly in the analysis of data (Sheiner and Grasela, 1991).

In order to extract the relative bioavailability information from the different parameters (such as CL/F and V_c/F), the relative bioavailability (F_{rel}) was included in the modelling process and fixed at one for the lowest dose solution concentration level while allowed to vary across the other dose levels tested. The sigmoidal model used to describe the relationship between the relative bioavailability and the concentration in the dosing solution indicated a 50% decrease in the F_{rel} parameter when the concentration in the dosing solution was increased from 1.25 to 40 mg/ml (Table 3). When using a dose solution concentration (CDS) of 5 mg/ml, a high degree of variability was observed as compared to the other levels (Fig. 3). The reason for this high variability is not clear, but it may be ascribed to the combination of a higher number of dogs on this level and a concentration in the dosing solution close to the estimated EC_{50} value (Table 3). Furthermore, the model indicated that at a given concentration level in the dosing solution (around 20 mg/ml), the bioavailability reaches its minimum and will not drop substantially below this point (Fig. 2). The curve representing the relationship between the relative bioavailability and the concentration in the dosing solution was found to be rather steep ($\gamma = 2.5$),

Table 4
Mean (±S.D.) pharmacokinetic parameters calculated after s.c. administration of four different dose levels to beagle dogs

DOSE (mg/kg)	N (NA)	C_{max} ng/ml	t_{max} (h)	FR (NA)	$t_{1/2}$ (k_{fast}) (h)
0.25 s.c.	3	25 ± 3	2.0 ± 0.1	0.53 ± 0.04	1.7 ± 0.3
0.5 s.c.	6	31 ± 6	3.7 ± 0.5	0.43 ± 0.03	4.6 ± 0.8
0.5 i.m.	3	64 ± 16	2.0 ± 0.1	0.44 ± 0.04	5.1 ± 0.2
1.0 s.c.	12	37 ± 15	12 ± 10	0.45 ± 0.07	3.7 ± 1.4
1.5 s.c.	3	62 ± 27	4.0 ± 0.1	0.44 ± 0.04	3.0 ± 0.3

C_{max}: the maximum plasma concentration based on NCA analysis of individually predicted data; t_{max}: the time at which C_{max} occurs; FR: the fraction of the dose absorbed via the fast path way (via k_{fast}); $T_{1/2}(k_{fast})$: The half-life based on the fast absorption rate constant.

which implicates a high degree of variability when using a dosing solution with a concentration around 9.17 mg/ml (F_{EC50}). Even though the estimated relative standard error (R.S.E.) on the gamma parameter was rather high (Table 3), an exclusion of this parameter resulted in a significant increase in the objective function value (Δ8.367), indicating the confidence interval of the parameter is non-symmetric.

The second most important covariate was the route of administration. When administered i.m. as opposed to s.c., degarelix entered into the systemic circulation more rapidly, its absorption rate at dose concentration of 0.5 mg/ml being about twice as high as after s.c. administration, i.e. 0.42 ± 0.07 and 0.19 ± 0.02, respectively. This was also reflected in more than twice as high C_{max} values when degarelix was administered i.m. rather than s.c. at the same dose level (Table 4). The time to reach the maximum concentration (t_{max}) was reduced from 3.7 h after s.c. administration to 2 h after i.m. administration (Table 4). To a minor extent, a low concentration in dosing solution also increased the fast absorption rate (k_{fast}) after s.c. administration, which is implemented in the model as a covariate relationship between the concentration in the dosing solution and k_{fast} (Fig. 2).

The use of mixed effects modelling to describe the pharmacokinetics of degarelix after s.c. and i.m. administration to beagle dogs provides us with a tool for controlling the release profile of degarelix. Even though the results apply to dogs, the depot must be expected to exhibit similar characteristics in all species, and hence the characteristics of the depot presented in the manuscript may also be expected to apply to clinical experiments. If high initial concentrations are necessary in a clinical setting, they may be achieved by choosing the i.m. rather than the s.c. route, the absorption will then be expected to take place more rapidly. Moreover, a low concentration in the dosing solution will increase the fraction absorbed (F_{rel}) and hence also the plasma concentration level. Although the release characteristics of the in-situ depot formed after injection of degarelix are complex, an understanding of these characteristics will provide us with a tool for controlling the degarelix plasma profile and thus optimize the treatment regimen of prostate cancer.

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