Pulmonary Delivery of Detirelix by Intratracheal Instillation and Aerosol Inhalation in the Briefly Anesthetized Dog

David B. Bennett,[1,6] Elizabeth Tyson,[2] Clinton A. Nerenberg,[3] Sylvia Mah,[1,4] Jennifer S. de Groot,[1] and Zvi Teitelbaum[1,5]

Received January 12, 1994; accepted March 15, 1994

Pulmonary delivery of the decapeptide detirelix was studied in briefly anesthetized dogs and the pharmacokinetics were examined following intravenous administration, intratracheal instillation, and aerosol inhalation. Detirelix administrations to the lung gave plasma profiles that were extended over two days, and that differed markedly from those of similarly sized peptides. Absorption from the lung after instillation was slow (T_{max} = 6.5 ± 3.6 h) with a relative bioavailability of 29 ± 10%. Administration of detirelix-containing aerosols resulted in similar plasma profiles as for administration by instillation. Compartmental and non-compartmental methods of pharmacokinetic analysis indicated no faster absorption from aerosols than from instilled solutions; an absorption rate limiting process may be an explanation. Plasma profiles were not affected by the use of detirelix liquid crystal favoring formulations or destabilizing formulations, and suggested that in situ liquid crystal formation was not an explanation for the slow absorption. No significant changes in pharmacokinetics or systemic uptake were observed during the five-month period of repeated pulmonary administrations. Histopathologic examination revealed the lungs to be essentially normal.

KEY WORDS: pharmaceutical aerosols; detirelix; LHRH antagonist; peptide delivery; pulmonary administration; pharmacokinetics; jet nebulizer.

INTRODUCTION

Detirelix (shown in Fig. 1) is an amphipathic decapeptide containing five D-amino acids [*N*-acetyl-D-3-(2-naphthyl)alanine-D-p-chlorophenylalanine-D-Trp-L-Ser-L-Tyr-D-*N,N'*-diethylhomoarginine-L-Leu-L-Arg-L-Pro-D-Ala-NH_2] and is one of the more potent luteinizing hormone-releasing hormone (LHRH) antagonists (1). Detirelix has very limited oral bioavailability (2); therefore, the potential utility of its pulmonary delivery was examined.

Pulmonary delivery of peptides and proteins is a clinically relevant route of administration for local and systemic action. The lungs' large absorptive area, thin alveolar epithelia, extensive vasculature, and avoidance of "first pass" hepatic metabolism make the lungs an attractive portal for systemic delivery. Absorption of high molecular weight lipid-insoluble compounds from the respiratory regions of the lungs may be mediated by a fluid phase transcytotic vesicular process (3,4) with size and charge exclusion capabilities (5–7). Systemic absorption of a peptide following pulmonary delivery will therefore be influenced by the peptide's physicochemical characteristics and deposition pattern. Residence time in the lung will also depend on a peptide's metabolic lability, potential for sequestration at membranes or within the surfactant, and mucociliary clearance of the peptide formulation.

Using detirelix as a model peptide, we examined its systemic absorption and pharmacokinetics in dogs following intravenous (i.v.) administration, intratracheal (i.t.) instillation, and aerosol inhalation (a.i.). Compartmental and non-compartmental methods of data analysis gave estimates of pharmacokinetic parameters. The effects of formulation variables were also studied to determine whether detirelix liquid crystals (8,9) affected pulmonary absorption.

MATERIALS AND METHODS

Formulations

Detirelix diacetate (molecular weight 1540.3 as the free base) was synthesized by the Institute of Organic Chemistry, Syntex Research. Buffer solutions were prepared using reagent grade or USP grade chemicals and purified water. UltraVent™ jet nebulizers were purchased from Mallinckrodt Medical, Inc. (Maryland Heights, MO).

Isotonic formulations of detirelix (1 mg/ml) in 5 m*M* acetate buffer (pH 5.2) with 3.1% mannitol were used for i.v. and i.t. dosings. Solutions for injection were sterile filtered (0.2 μm) prior to use. Rinse solutions for i.t. instillation consisted of physiological saline or isotonic glycine buffer (5 m*M* glycine, 4% mannitol, pH 7.4). Formulations for nebulization consisted of detirelix (4 mg/ml) in isotonic phosphate buffered saline (PBS, 5 m*M* phosphate, 0.9% NaCl, pH 7.4) or isotonic glycine buffer. All formulations were stored below 5°C, warmed to room temperature before use, and assayed by HPLC (10) to confirm stability. Formulations were prepared such that liquid crystalline gels of detirelix would not form under the storage conditions and this was confirmed by visual inspection of each formulation before use.

Animals

Two male and two female adult laboratory-bred mongrel dogs, 14 to 17 months of age (12.5 to 14.6 kg), were used in a series of i.v., i.t., and a.i. studies over a period of five months. The dogs were housed in an A.A.L.A.S. approved facility and a registered veterinary technician monitored anesthesia, performed intubation, and was present at each drug administration. The dogs were fed at least once daily and allowed free access to water; however, food was withheld overnight prior to each dosing. Dogs were weighed prior to

[1] Center for Drug Delivery Research, Syntex Research
[2] Institute of Pathology, Toxicology, and Metabolism, Syntex Research
[3] Institute of Analytical Research, Syntex Research
[4] Present address: Amgen, Inc., Thousand Oaks, CA
[5] Present address: Israel Institute of Biological Research, P.O. Box 19, Ness Ziona 70450, Israel
[6] To whom correspondence should be addressed:
David B. Bennett
Syntex Research
3401 Hillview Ave.
Palo Alto, CA 94304

0724-8741/94/0700-1048$07.00/0

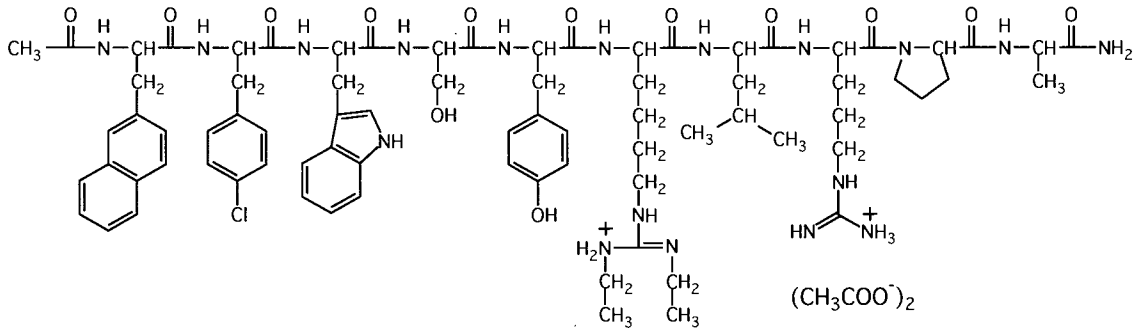

Figure 1. The chemical structure of detirelix.

each detirelix administration and the doses were adjusted for body weight. At least one week between detirelix administrations served as a washout period, verified by the drawing of pre-dose plasma samples. All procedures involving animals were approved by the Syntex Institutional Animal Care and Use Committee.

Each dog was anesthetized with valium (1 mg/kg) and ketamine (10 mg/kg) by injection into a foreleg cephalic vein prior to each detirelix administration. Detirelix was then administered as described below. Breathing was unassisted and spontaneous at all times. All dogs regained consciousness and were fully mobile within one hour after induction of anesthesia.

Intratracheal Instillation

Dogs were intubated with a cuffed Rüsch endotracheal tube (ET, 32 × 1 cm i.d.) immediately after induction of anesthesia. The distal end of the ET extended approximately 15 cm beyond the larynx and to within approximately 5 cm of the tracheal bifurcation as determined by comparison of the ET length with anatomical landmarks. After inflation of the ET cuff, the dogs were placed in a supine position and a T-shaped union was attached to the external end of the ET to facilitate instillation. The T-shaped union had previously been modified such that one of the three ports was covered by a breathing circuit filter (model #BB-50T, Pall Biomedical Products Corp., East Hills, NY) and another port was covered by a rubber septum. The rubber septum was pierced by a 15 gauge luer stub adapter (model #7560, Becton Dickinson) and a three-way stopcock was attached to the external part of the leur stub adapter. The internal arm of the leur stub adapter was connected to a length of polyethylene tubing (~48 cm × 1.67 mm i.d.) which had been previously cut to extend exactly to the distal end of the ET. The internal dead volume of the stopcock, the leur stub adapter, and the polyethylene tubing was about 1.3 ml. Upon attachment of the T-shaped union to the ET the polyethylene tubing extended coaxially within the ET to the distal end.

Instillations were accomplished in a two step manner: the detirelix solution (0.3 to 1.3 ml) was injected into the polyethylene tubing through the stopcock and remained within the polyethylene tubing until dosing was completed by the rapid instillation of a 10-ml rinse solution (saline, unless otherwise noted) through the polyethylene tubing. The rinse was given in two portions, each coordinated with inspiration, and complete delivery of the dose to the lungs was assumed. Dogs were extubated within 10 to 15 minutes and they regained full consciousness within another 30 to 45 minutes after detirelix instillation.

The first set of detirelix dosings consisted of a random crossover study between i.v. (30 μg/kg) and i.t. (80 μg/kg) administrations to determine whether delivery of detirelix directly to the lungs produced anaphylaxis. After no adverse reactions were observed in the initial administrations, the subsequent i.t. administrations of detirelix at 40 and 160 μg/kg were performed in a randomized crossover manner to determine pharmacokinetic linearity. The remainder of the detirelix dosings were performed sequentially; however, the 80 μg/kg formulation was re-administered (i.t.) at the conclusion of the study period as a check for sequence-induced pharmacokinetic changes.

Aerosol Delivery

Dogs were anesthetized and intubated as in the i.t. administrations; however, the external end of the ET was connected directly to the outlet of the UltraVent nebulizer. The nebulizer reservoir was filled with 4 ml of the appropriate detirelix formulation and the aerosol was generated with O_2 delivered at a flow rate of 6 l/min. The nebulizer was partially immersed in a warm water bath during nebulization to minimize potentially undesirable respiratory effects due to inhalation of a cold aerosol. Nebulization was stopped after 15 minutes and the nebulizer was disconnected from the ET; time zero was recorded and the dogs regained consciousness normally. The nebulizer was weighed before and after aerosol administration.

Blood Collection and Detirelix Assay

Blood samples, drawn by direct venipuncture up to 32 h (i.v.) or 48 h (i.t. and a.i.) after detirelix administration, were transferred to heparinized tubes and the plasma was immediately separated by centrifugation. Plasma samples were stored at −20°C until the detirelix concentration was determined by a specific radioimmunoassay (lower limit of quantitation ca. 0.2 ng/ml) (11). Reported concentrations were the average of duplicate runs; samples whose concentration exceeded the standard curve maximum were diluted and reassayed.

Necropsy and Tissue Histopathology

Dogs were euthanized by sodium pentobarbital over-

dose one week after the final detirelix administration. The lungs and trachea were immediately removed and fixed by gentle gravimetric instillation of approximately 20 to 30 ml formalin (10% formaldehyde in neutral sodium acetate buffer) and then fully immersed in the fixative. The lungs were sectioned, stained with hematoxylin-eosin, Gamori's reticulum, or Masson's trichrome, and were later examined for abnormalities in microscopic anatomy.

Pharmacokinetic Analyses

Pharmacokinetic parameters were determined using statistical moment analysis and nonlinear least-squares regression analysis. The area under the plasma concentration-time curve (AUC) and the area under the first moment of the plasma concentration-time curve (AUMC) were calculated by the trapezoidal method, and the areas after the last sampling time were obtained by extrapolation (12). The elimination half-life ($t_{1/2,\beta}$) was calculated using the slope of the terminal log-linear portion of the plasma profile. Bioavailability (F) was determined using the dose-normalized AUC, and the systemic clearance, Cl_s, was calculated using the i.v. dose ($D_{i.v.}$):

$$Cl_s = D_{i.v.} / AUC \quad (1)$$

T_{max} was the time to the sample with the maximum plasma detirelix concentration (C_{max}). T_{max} and C_{max} were used as qualitative pharmacokinetic parameters due to the limited number of sampling times.

Compartmental pharmacokinetic analysis employed a two-compartment open model for i.v. and a three-compartment open model, shown in Fig. 2, for i.t. and a.i. plasma detirelix profiles. The i.v. and pulmonary delivery data were fit by weighted ($1/y^2$) and unweighted nonlinear least-squares regression analyses, respectively, using MINSQ (MicroMath, Salt Lake City, UT). Regression analysis of an individual dog's i.v. data involved fitting to an equation of the form:

$$C_p = A'e^{-\alpha' t} + B'e^{-\beta' t} \quad (2)$$

where C_p is the plasma detirelix concentration and A', B', α', and β' are constants (13). The curve fitting resulted in the determination of each dog's plasma elimination rate constant (k_{el}) and distribution rate constants (k_{12} and k_{21}). The steady state volume of distribution (V_{ss}) was determined from the i.v. data and the two-compartment model (13) by use of the following equation:

$$V_{ss}\ (\text{compartmental}) = D_{i.v.}(A'/\alpha'^2 + B'/\beta'^2) / (A'/\alpha' + B'/\beta')^2 \quad (3)$$

The three-compartment model (Fig. 2) resulted in the estimation of the rate constant for pulmonary absorption (k_{abs}) and the rate constant representing the combined effects of all non-absorptive elimination processes in the lung ($k_{el,2}$). Its analytical solution gave the following triexponential expression:

$$C_p = (D_{pul}k_{abs}/V)\cdot[Ae^{-\alpha(t-T_{lag})} + Be^{-\beta(t-T_{lag})} + Ce^{-\gamma(t-T_{lag})}] \quad (4)$$

The terms are defined in the glossary of this report. A lag time (T_{lag}) of up to fifteen minutes was incorporated to facilitate a more accurate estimation of k_{abs}; the finite time for detirelix to cross the air-blood barrier was the physiological basis for inclusion of T_{lag}. The k_{el}, k_{12} and k_{21} values obtained from each dog's i.v. data were used as initial estimates in fitting the three-compartment model to that individual dog's pulmonary data. A stepwise curve fitting strategy was employed for each pulmonary data set, whereby only one of the parameters k_{abs}, $k_{el,2}$, or T_{lag} was initially selected to vary while the other two parameters were restricted to their initial estimates. Once the curve fitting procedure converged, the restriction was removed for an additional parameter and the curve fitting was repeated. This stepwise curve fitting was repeated until convergence was obtained with k_{abs}, $k_{el,2}$, and T_{lag} all selected to freely vary. A final refinement of the curve fitting was obtained when k_{el}, k_{12}, and k_{21} were also permitted to vary within their respective 95% confidence intervals; confidence intervals were calculated based on the curve fitting of the i.v. data of all dogs. The final outcome of this procedure was independent of the order in which the restrictions on the variation of k_{abs}, $k_{el,2}$, and T_{lag} were removed. The goodness of fit was determined using the MINSQ model selection criterion (a modified Akaike information criterion).

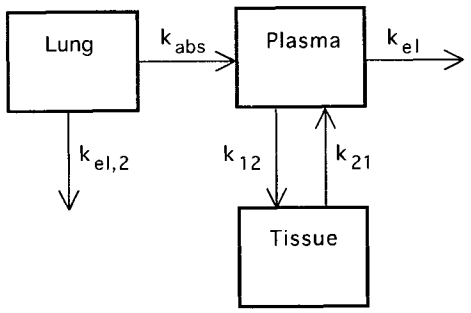

Figure 2. Three-compartment open model for pulmonary administration with lung, plasma (central), and tissue (peripheral) compartments. The first-order rate constants k_{abs}, $k_{el,2}$, k_{el}, k_{12}, and k_{21} represent systemic uptake from the lung, non-absorptive clearance from the lung, elimination from the plasma, and (re-)distribution between the plasma and tissue compartments, respectively.

Statistical moment analysis is a non-compartmental method for estimating pharmacokinetic parameters (14). The mean residence time (MRT) of the drug in the body was defined (14) as:

$$MRT = \int_0^\infty t \cdot C_p\, dt / \int_0^\infty C_p\, dt = AUMC / AUC \quad (5)$$

and was calculated for each dog based on its individual i.v. and pulmonary data; it was assumed that $MRT_{i.v.}$ did not change over the five month course of this study. A non-compartmental based V_{ss} was calculated for the i.v. data based on the method of Benet and Galeazzi (12), where:

$$V_{ss}\ (\text{non-compartmental}) = Cl_s \cdot MRT_{i.v.} \quad (6)$$

Comparison of V_{ss} (compartmental) with V_{ss} (non-compartmental) served as a qualitative assessment of the validity of the two-compartment model.

The MRT for pulmonal input involves an absorptive component, the mean absorption time (MAT) (14), where:

$$MAT = MRT_{pul} - MRT_{i.v.} \quad (7)$$

If the absorption process is assumed to be first-order, an absorption rate constant (k'_{abs}) can be estimated by the reciprocal of MAT. However, if a competing first-order elimination process occurs at the input site, the absorption rate constant is overestimated and a rate constant for the competing process (k'_{el}) must be incorporated (14). The relationship becomes:

$$MAT^{-1} = k'_{abs} + k'_{el} \approx k_{clear} \quad (8)$$

We shall refer to the reciprocal of MAT as an approximation of the rate constant for total clearance of detirelix from the lung (k_{clear}).

Statistical Analysis

The statistical analysis of multiple data sets was performed using a one-factor analysis of variance and pairwise comparisons were performed using the two tailed t-test; $p < 0.05$ were considered significant. It was assumed that there were no sequence related effects due to non-randomization of some studies. Since the same four dogs were used throughout the study, each dog served as its own bioavailability control. Reported pharmacokinetic values represented the mean of all four dogs ± one standard deviation, unless otherwise specified.

RESULTS AND DISCUSSION

In a preliminary study (15) detirelix was administered via i.v. and i.t. modes to surgically prepared and deeply (pentobarbital) anesthetized dogs; however, monitoring for more than eight hours was impractical and, consequently, incomplete plasma profiles were obtained. In our study reported here, an animal model that permitted long-term monitoring using briefly anesthetized dogs was utilized to facilitate dosing and to allow for plasma sampling from conscious dogs over the course of several days. No anaphylaxis or other adverse effects of dosing were observed at any time during this study. While the use of briefly anesthetized dogs facilitated pulmonary dosing and presented an alternative to the use of tracheostomized dogs (16), the effects of the anesthetics on the absorption and clearance of detirelix were not determined.

Intravenous Administration

A biphasic plasma profile was observed following the i.v. administration of detirelix, shown in Fig. 3, and the corresponding non-compartmental pharmacokinetic parameters are listed in Table I. Biphasic plasma profiles were also observed for i.v. detirelix in rats and male cynomolgus monkeys (2). Cl_s was found to be 0.97 ± 0.38 ml min^{-1} kg^{-1}, which was comparable to that observed in male cynomolgus monkeys (2), and $t_{1/2,\beta}$ was 7.9 ± 1.4 h. The mean plasma elimination rate constant (k_{el}) determined by the two-compartment model was found to be 0.34 ± 0.09 h^{-1}, as listed in Table II, and corresponds to a plasma elimination half-life of 2.1 h, a value that was intermediate to those determined for detirelix in the rat and monkey (2). The rate constants characterizing the distribution between the plasma and peripheral compartments (k_{12}, k_{21}) were each estimated to be 0.16 ± 0.06 h^{-1}. The compartmental and non-compartmental V_{ss} were determined to be 390 ± 80 ml/kg and 340 ± 90 ml/kg, respectively, and suggested agreement between the two pharmacokinetic methods. Also, no significant differences were observed between the pharmacokinetic parameters of the male and the female dogs for any i.v. or pulmonary administrations.

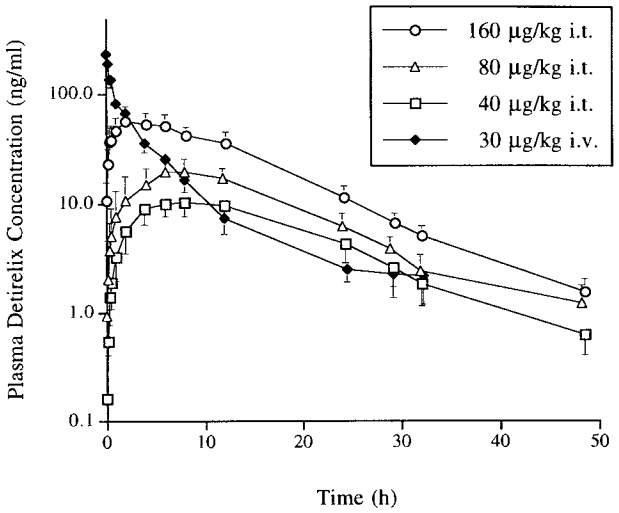

Figure 3. Plasma detirelix profiles following i.v. and i.t. delivery of various doses to briefly anesthetized and spontaneously breathing dogs. Each instillation was performed with a saline rinse to normalize instilled volume and to ensure complete delivery of dose. Values represent the mean ± standard error (n = 4).

Intratracheal Instillation

Instillations were performed with a 10-ml rinse to provide a more constant total volume among the administrations and to minimize the potential differences in spreading and deposition that may occur upon instillation of various fluid volumes. The total instilled fluid volume was of similar proportion to body weight as was delivered to rodents in other studies (17). Plasma profiles after instillation of detirelix at 40, 80, and 160 μg/kg are shown in Fig. 3. The non-compartmental pharmacokinetic parameters listed in Table I indicated a mean bioavailability for all i.t. administrations of 29 ± 10% relative to i.v.. The average $t_{1/2,\beta}$ was 8.2 ± 0.9 h and compared well to the i.v. value. No significant differences were found among all the i.t. dosings for MRT, MAT, k_{el}, dose normalized C_{max}, or dose normalized AUC; however, while not significant among i.t. dosings, T_{max} was decreased at the highest dose (160 μg/kg). These data suggested the pharmacokinetics of detirelix administered by i.t. instillation were linear in the dose range examined.

The i.t. plasma profiles showed an extended absorption phase prior to a plateau near maximal plasma levels. T_{max} for detirelix averaged 6.5 ± 3.6 h and was extremely long compared with values obtained for similarly sized peptides (18,19). Clearly, factors other than molecular weight must be considered in a prediction of T_{max}.

The MAT of instilled detirelix averaged 8.8 ± 2.1 h and was nearly fivefold greater than that of insulin in the rat (19). The calculated value of k_{clear} was 0.12 ± 0.03 h^{-1} and correlated well with interspecies predictions for solute clear-

Table I. Summary of Non-Compartmental Pharmacokinetic Parameters (mean ± standard deviation).

Route	Dose (μg/kg)	Vehicle[a]	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0\to\infty}$ (ng·h/ml)	MRT (h)	MAT (h)	k_{clear} (h^{-1})	*F* (%)
i.v.	30	—	—	—	580 ± 210	6.3 ± 0.7	—	—	100
i.t.	40	saline	8.0 ± 3.3	10 ± 5	230 ± 130	16 ± 2	10 ± 1	0.098 ± 0.013	27 ± 7
i.t.	80[b]	saline	9.4 ± 2.9	20 ± 12	400 ± 260	16 ± 1	9.5 ± 0.6	0.11 ± 0.01	26 ± 9
i.t.	160	saline	3.5 ± 3.0	64 ± 29	970 ± 480	13 ± 2	6.5 ± 1.6	0.16 ± 0.03	34 ± 15
i.t.	80	glycine	5.8 ± 4.1	21 ± 9	390 ± 170	16 ± 4	9.5 ± 3.0	0.11 ± 0.03	28 ± 7
i.t.	80[c]	saline	5.3 ± 1.9	26 ± 19	470 ± 360	14 ± 2	7.8 ± 1.5	0.13 ± 0.03	28 ± 14
	average (i.t.)		6.5 ± 3.6	—	—	15 ± 2	8.8 ± 2.1	0.12 ± 0.03	29 ± 10
a.i.	—[d]	PBS	8.4 ± 2.7	12 ± 5	310 ± 110	19 ± 3	13 ± 2	0.080 ± 0.012	—
a.i.	—[d]	glycine	6.7 ± 1.5	11 ± 2	270 ± 70	20 ± 5	14 ± 4	0.077 ± 0.019	—
	average (a.i.)		7.6 ± 2.2	—	290 ± 90	19 ± 4	13 ± 3	0.078 ± 0.015	—

[a] rinse solution for i.t., formulation vehicle for a.i.; [b] initial i.t. administration; [c] administration performed five months after beginning of study; [d] reliable estimate of inhaled dose unavailable.

ance from the lung based on molecular weight, as suggested by Effros and Mason (20). The MRT and MAT of detirelix following instillation in the briefly anesthetized dog were similar to those obtained using conscious sheep (21).

The two-compartment pharmacokinetic model for the i.v. dosing was extended to include a third compartment describing the peptide depot in the lung lumen (Fig. 2). The three-compartment model enabled estimation of the rates of detirelix absorption (k_{abs}) and clearance from the lung ($k_{el,2}$) and are shown in Table II. The MINSQ model selection criterion established the three-compartment model to be superior to the three-compartment model without T_{lag}, a two-compartment model, and weighted two- and three-compartment models. The results of the curve fitting for representative i.t. and a.i. data sets are shown in Fig. 4. k_{abs} and $k_{el,2}$ values did not vary significantly among the i.t. doses.

Our absorption rate constants for i.t. detirelix are two orders of magnitude less than those reported for the clearance of liquids from canine lungs (22). This indicates that the systemic absorption rate constants (k_{abs}) calculated from our data were not substantially influenced by instillation with rinse solutions, and detirelix was therefore not appreciably transported across the air-blood barrier as a result of bulk fluid absorption (22). The incomplete bioavailability of i.t. detirelix was a reflection of the substantially greater calculated values of $k_{el,2}$ as compared to k_{abs} (i.e., elimination in the lung was greater than absorption across the air-blood barrier). $k_{el,2}$ may represent the combined rate of in situ metabolism (including phagocytosis by alveolar macrophages), the mucociliary clearance from non-alveolar deposition sites, and other non-absorptive pathways. And while detirelix is stable in bronchoalveolar lavage fluid in vitro (10), the actual degree of enzymatic degradation in situ is unknown. It is noteworthy that, although derived from different models, the sum of k_{abs} and $k_{el,2}$ is very nearly equal to k_{clear} in equation 8.

A decrease in AUC was reported following fourteen days of single-dose, intratracheal aerosol administration of leuprolide acetate to dogs (16). Our data indicated no significant difference in any pairwise comparison of detirelix pharmacokinetic parameters (Tables I and II) between the original instillation of detirelix (80 μg/kg) and instillation of the same dose five months later; the plasma profiles are compared in Fig. 5.

The relatively slow pulmonary absorption of detirelix was initially believed to be related to its ability to form liquid crystalline gels in concentrated aqueous solutions (8). This unique phenomenon was reported to be accelerated in the presence of anions such as HPO_4^{-2} and Cl^-, and inhibited by hydrophilic zwitterions such as glycine (9). Therefore, the composition of the i.t. rinse solution was varied from the

Table II. Summary of Compartmental Pharmacokinetic Parameters (mean ± standard deviation).

route	dose (μg/kg)	vehicle[a]	k_{abs} (h^{-1})	$k_{el,2}$ (h^{-1})	k_{el} (h^{-1})
i.v.	30	—	—	—	0.34 ± 0.09
i.t.	40	saline	0.0023 ± 0.0015	0.11 ± 0.01	0.17 ± 0.07
i.t.	80[b]	saline	0.00077 ± 0.00030	0.11 ± 0.02	0.25 ± 0.24
i.t.	160	saline	0.0016 ± 0.0008	0.54 ± 0.47	0.26 ± 0.23
i.t.	80	glycine	0.0020 ± 0.0021	0.15 ± 0.10	0.30 ± 0.21
i.t.	80[c]	saline	0.0030 ± 0.0005	0.15 ± 0.09	0.29 ± 0.21
	average (i.t.)		0.0014 ± 0.0013	0.22 ± 0.26	0.23 ± 0.18
a.i.	—[d]	PBS	0.0016 ± 0.0021	0.095 ± 0.021	0.12 ± 0.03
a.i.	—[d]	glycine	0.0031 ± 0.0048	0.096 ± 0.028	0.17 ± 0.11
	average (a.i.)		0.0024 ± 0.0035	0.096 ± 0.023	0.15 ± 0.08

[a] rinse solution for i.t., formulation vehicle for a.i.; [b] initial i.t. administration; [c] administration performed five months after beginning of study; [d] reliable estimate of inhaled dose unavailable.

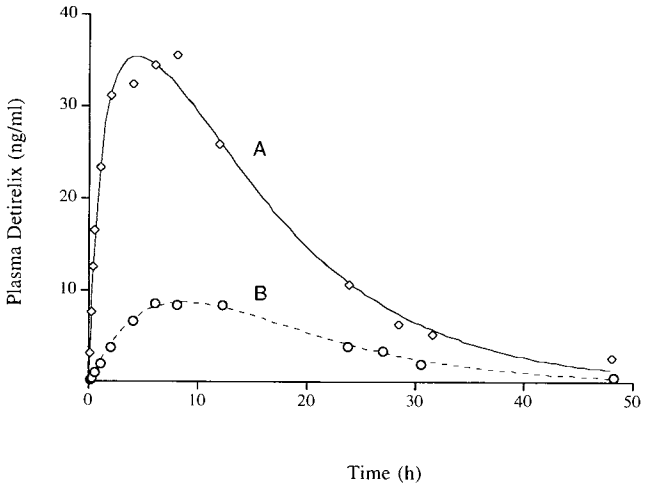

Figure 4. Representative comparison of actual plasma detirelix concentrations with profiles simulated by nonlinear curve fitting to the three-compartment pharmacokinetic model using MINSQ:
A, intratracheal instillation, –◇– (80 μg/kg, saline rinse, dog #4);
B, aerosol inhalation, --○-- (PBS vehicle, dog #1).

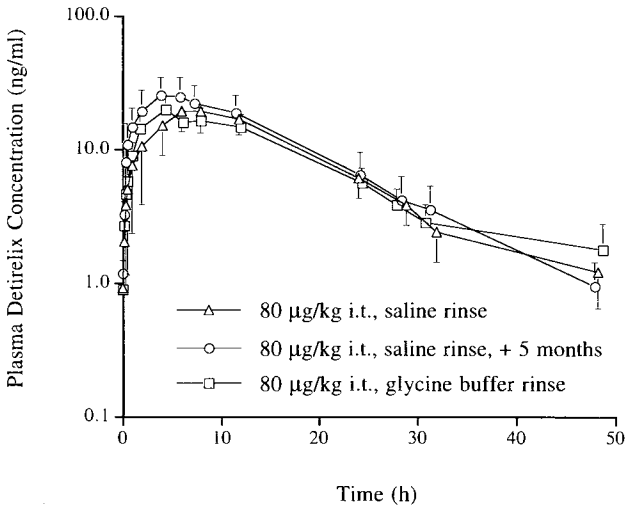

Figure 5. Comparison of plasma detirelix profiles after i.t. delivery using liquid crystal favoring (physiological saline) and liquid crystal destabilizing (glycine buffer) rinse solutions. Five months after the initial study, an identical dose of detirelix was instilled with a saline rinse. Values represent the mean ± standard error (n = 4).

liquid crystal favoring saline to the liquid crystal destabilizing glycine buffer to test whether possible in situ formation of liquid crystals affected absorption kinetics. Instillation of detirelix at 80 μg/kg with the chloride-free glycine buffer rinse resulted in a plasma profile (Fig. 5) similar to that obtained with the saline rinse. Also, the pharmacokinetic parameters (Table I and Table II) obtained with the glycine buffer regimen were not significantly different when compared in a pairwise manner to each of the two studies which used a saline rinse and the same dose of detirelix. The results suggested that in situ liquid crystal formation was not a factor in limiting absorption from the lung.

Aerosol Inhalation

During aerosol deliveries the nebulizers were run to near dryness and the weight loss after administration averaged 2.30 ± 0.18 g. Although the weight loss was relatively uniform among the aerosol deliveries, bioavailabilities were not calculated because of the error inherent in estimating the dose delivered to the lung (i.e., solvent evaporation and aerosol deposition in the ET).

Plasma profiles resulting from the inhalation of detirelix aerosols are plotted in Fig. 6 and are similar to those observed after i.t. administration. The $t_{1/2,\beta}$ and T_{max} averaged 10 ± 2 h and 7.6 ± 2.2 h, respectively, and were similar to the i.t. deliveries. As with instillation, this exceptionally long T_{max} was much greater than those observed for the similarly sized peptides leuprolide acetate (16) and cyclosporine A (23) following aerosol delivery to dogs. The MAT of detirelix after a.i. delivery was 13 ± 3 h and resulted in an average k_{clear} of 0.078 ± 0.015 h^{-1}. The slightly decreased k_{clear} relative to i.t. dosings corresponded to a similar decrease in the average k_{abs} and average $k_{el,2}$ (Table II). As with the i.t. administrations, the sum of k_{abs} and $k_{el,2}$ was similar to k_{clear} for inhalation of detirelix aerosols.

No significant differences were observed in pairwise comparisons of pharmacokinetic parameters (Table I and Table II) between the use of aerosols generated from either liquid crystal favoring (PBS) or destabilizing (glycine buffer) solutions. Similar to i.t. deliveries, these data also suggested that the prolonged absorption of detirelix from the lung was not explained by in situ liquid crystal formation.

Schanker and coworkers (17) reported a twofold increase in the rate of pulmonary absorption following inhalation as compared with instillation. However, detirelix exhibited no increase in the calculated rate of absorption (k_{abs}) when administered by aerosol versus instilled solution. The existence of an absorption rate limiting process, independent of molecular size and liquid crystal effects, was a possible explanation. For example, detirelix associated strongly with phosphatidylcholine-containing liposomes (10); the major

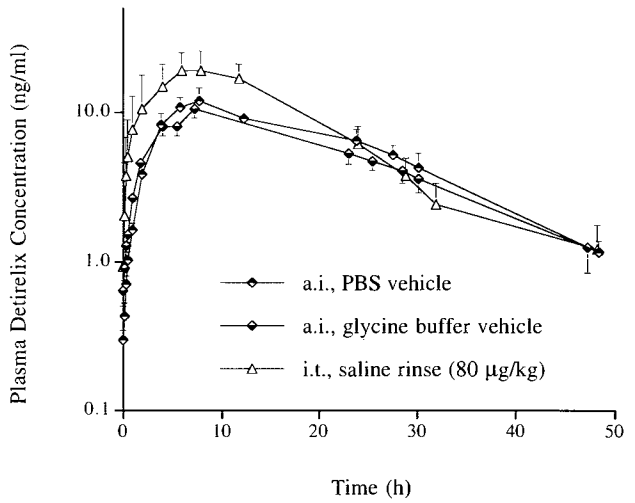

Figure 6. Plasma detirelix profiles following inhalation of aerosols generated from liquid crystal favoring (PBS) and liquid crystal destabilizing (glycine buffer) vehicles. The original i.t. (80 μg/kg) profile is included for comparison. Values represent the mean ± standard error (n = 4).

component of pulmonary surfactant is phosphatidylcholine (24). Consequently, association of detirelix with pulmonary surfactant, or with other lung tissue components, may have resulted in its prolonged absorption from the lung (6,7).

Lung Histopathology

At the conclusion of the study the lungs of all four dogs were found to be essentially normal with the exception of focal and multifocal minimal inflammatory or minimal hyperplastic changes. Notably, detirelix was reported to elicit a mild histamine release when injected subcutaneously (1); this may also have occurred in the dogs' lungs and contributed to the minimal changes in microscopic anatomy observed. However, as no control dogs were compared, it is not known whether the changes were due to the detirelix or the administration techniques. Adjei and coworkers (16) also found mild microscopic and inflammatory reactions in the lungs of beagle dogs after i.t. delivery of leuprolide.

CONCLUSIONS

Detirelix was administered with no untoward effects to the lungs of briefly anesthetized dogs by instillation and inhalation. The resulting plasma profiles were extended over two days and differed markedly from those of similarly sized peptides. Pharmacokinetic analysis indicated similar rates of absorption from aerosols and instilled solutions, and may be attributed to our use of solutions to rinse the drug from the i.t. delivery device (giving a deeper and more uniform drug deposition), or to the existence of an absorption rate limiting process. The results suggested the absorption rate was not limited by the in situ formation of detirelix liquid crystalline gels. The cause of the extended absorption times is uncertain, but may be related to the physicochemical properties of the peptide.

ACKNOWLEDGMENTS

The authors thank Peggy Murphy-Hackley, Steve Hall, and Martin Brovarny for their expert assistance with the animals; Susan Winslow for tissue necropsy and Gary R. Cain for evaluation of lung tissue sections; Steve Terao, Frances Chu, and Joanne LaFargue for RIA analyses; Susan Musick for editorial assistance; Hans Schreier for helpful discussions; and Lynda Sanders for her support of this project. The authors also acknowledge the reviewers of this manuscript for suggesting valuable additions and changes.

NOTATIONS

The following abbreviations were used in this report and are defined as follows:

α	$= k_{abs} + k_{el,2}$
A	$= (k_{21} - \alpha)/[(\beta - \alpha)(\gamma - \alpha)]$
A.A.L.A.S.	American Association of Laboratory Animal Science
β	$= (k_{21} + k_{12} + k_{el} + R)/2$
B	$= (k_{21} - \beta)/[(\alpha - \beta)(\gamma - \beta)]$
γ	$= (k_{21} + k_{12} + k_{el} - R)/2$
C	$= (k_{21} - \gamma)/[(\alpha - \gamma)(\beta - \gamma)]$
D_{pul}	pulmonary dose
R	$= [(k_{21} + k_{12} + k_{el})^2 - 4 \cdot k_{el} \cdot k_{21}]^{1/2}$
t	time
V	volume of distribution in the lung lumen

REFERENCES

1. S. N. Pavlou, G. B. Wakefield, D. P. Island, P. G. Hoffman, M. E. Le Page, R. L. Chan, C. A. Nerenberg, and W. J. Kovacs. Suppression of pituitary-gonadal function by a potent new luteinizing hormone-releasing hormone antagonist in normal men. *J. Clin. Endocrinol. Metab.* 64:931–936 (1987).
2. R. L. Chan, W. Ho, A. S. Webb, J. La Fargue, and C. A. Nerenberg. Disposition of detirelix, a potent luteinizing hormone-releasing hormone antagonist, in rats and monkeys. *Pharm. Res.* 5:335–340 (1988).
3. K. G. Bensch and E. A. M. Dominguez. Studies on the pulmonary air-tissue barrier. Part IV: Cytochemical tracing of macromolecules during absorption. *Yale J. Biol. Med.* 43:236–241 (1971).
4. J. Gil. Number and distribution of plasmalemmal vesicles in the lung. *Fed. Proc.* 42:2414–2418 (1983).
5. S. J. Enna and L. S. Schanker. Absorption of saccharides and urea from the rat lung. *Am. J. Physiol.* 222:409–414 (1972).
6. D. Simionescu and M. Simionescu. Differentiated distribution of the cell surface charge on the alveolar-capillary unit. *Microvascular Res.* 25:85–100 (1983).
7. M. C. Williams. Endocytosis in alveolar type II cells: Effect of charge and size of tracers. *Proc. Natl. Acad. Sci. USA* 81:6054–6058 (1984).
8. M. F. Powell, L. M. Sanders, A. Rogerson, and V. Si. Parenteral peptide formulations: Chemical and physical properties of native luteinizing hormone-releasing hormone (LHRH) and hydrophobic analogues in aqueous solution. *Pharm. Res.* 8:1258–1263 (1991).
9. J. Fleitman, M. F. Powell, L. M. Sanders, and V. C. Si. Peptide liquid crystals: Inverse correlation of kinetic formation and thermodynamic stability in aqueous solutions. *Pharm. Res.* (Submitted).
10. D. B. Bennett, E. Tyson, S. Mah, J. S. de Groot, S. Terao, S. Hegde, and Z. Teitelbaum. Sustained delivery of detirelix after pulmonary administration of liposomal formulations. *J. Contr. Rel.* (Submitted).
11. C. Nerenberg, J. La Fargue, A. Webb, R. Chan, and S. Kushinsky. Radioimmunoassay of detirelix ([*N*-Ac-D-Nal(2)^1, D-p-Cl-Phe^2, D-Trp^3, D-hArg(Et)$_2^6$, D-Ala^{10}]-luteinizing hormone-releasing hormone) in plasma or serum. *J. Immunoassay* 9:245–255 (1988).
12. L. Z. Benet and R. L. Galeazzi. Noncompartmental determination of the steady-state volume of distribution. *J. Pharm. Sci.* 68:1071–1074 (1979).
13. M. Gibaldi and D. Perrier. *Pharmacokinetics*, Marcel Dekker, Inc., New York, 1982, pp. 103-109.
14. D. J. Cutler. Theory of the mean absorption time, an adjunct to conventional bioavailability studies. *J. Pharm. Pharmacol.* 30: 476–478 (1978).
15. D. Bennett, J. La Fargue, C. Nerenberg, R. Maskiewicz, L. Wood, and Z. Teitelbaum. Sustained plasma levels of detirelix following intratracheal instillation to anesthetized dogs. *Pharm. Res.* 9:S201 (1992).
16. A. Adjei, R. Doyle, M. Pratt, R. Finley, and E. Johnson. Bioavailability of leuprolide following intratracheal administration to beagle dogs. *Int. J. Pharm.* 61:135–144 (1990).
17. L. S. Schanker, E. W. Mitchell, and R. A. Brown. Species comparison of drug absorption from the lung after aerosol inhalation or intratracheal injection. *Drug Metab. Dispos.* 14:79–88 (1986).
18. H. G. Folkesson, B. R. Weström, and B. W. Karlsson. Permeability of the respiratory tract to different-sized macromolecules after intratracheal instillation in young and adult rats. *Acta Physiol. Scand.* 139:347–354 (1990).
19. F.-Y. Liu, Z. Shao, D. O. Kildsig, and A. K. Mitra. Pulmonary

delivery of free and liposomal insulin. *Pharm. Res.* **10**:228–232 (1993).
20. R. M. Effros and G. R. Mason. Measurements of pulmonary epithelial permeability in vivo. *Amer. Rev. Resp. Dis.* **127**:S59–S65 (1983).
21. H. Schreier, K. J. McNicol, D. B. Bennett, Z. Teitelbaum, and H. Derendorf. Pharmacokinetics of detirelix following intratracheal instillation and aerosol inhalation in the unanesthetized awake sheep. *Pharm. Res.* 11(7):1056–1059 (1994).
22. Y. Berthiaume, V. C. Broaddus, M. A. Gropper, T. Tanita, and M. A. Matthay. Alveolar liquid and protein clearance from normal dog lungs. *J. Appl. Physiol.* **65**:585–593 (1988).
23. G. J. Burckart, R. Dowling, M. Zenati, S. A. Yousem, R. Venkataramanan, and B. P. Griffith. Cyclosporine administration by aerosol. *J. Clin. Pharmacol.* 29:860 (1989).
24. R. J. King and J. A. Clements. Surface active materials from the dog lung. I. Method of isolation. *Am. J. Physiol.* **223**:707–714 (1972).