Pharmacokinetics of Detirelix Following Intratracheal Instillation and Aerosol Inhalation in the Unanesthetized Awake Sheep

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The unanesthetized awake sheep was employed as large animal model for the determination of bioavailability and pharmacokinetics following the pulmonary instillation of the decapeptide detirelix. After intratracheal administration of a 80 μg/kg dose, the average $t_{1/2}$ of elimination was 9.8 ± 1.3 hours (n = 5) which was similar to the elimination kinetics of a 30 μg/kg i.v. dose (7.2 ± 2.9 hours). Mean residence time (MRT) was prolonged to 10.3 ± 2.0 hours vs. 2.7 ± 0.8 hours i.v., and mean absorption time (MAT) was calculated to be 7.5 ± 1.8 hours. Maximum plasma levels (c_{max}) of 9.2 ng/ml were reached after 2 hours. The average bioavailability was 9.8 ± 3.9% of the dose. The pharmacokinetic profile was found to be similar after aerosol administration. It was concluded that detirelix was absorbed systemically when administered by pulmonary instillation or aerosolization and that the unanesthetized awake sheep is a suitable model to study resulting drug profiles.

INTRODUCTION

The decapeptide detirelix [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] is a highly potent luteinizing hormone-releasing hormone (LHRH) antagonist (1) (see also accompanying paper by Bennett et al. (2)).

Delivery of proteins and peptides by routes other than parenteral, particularly by the nasal (3) and oral route (4), is still an elusive goal. Both the (mature) intestinal as well as the nasal mucosa are practically impermeable for macromolecules, resulting in therapeutically irrelevant bioavailabilities of <1% in most cases (3,4). While formulations employing detergents enhance nasal absorption markedly (3), the long-term effects of chronic detergent exposure on mucosal membranes are unknown and continue to hamper developments in this direction.

Recently, pulmonary delivery via intratracheal instillation or aerosol inhalation has been explored as a noninvasive alternative (5). Use of the lung as portal of entry for systemic peptide or protein delivery appears attractive due to the lung's large surface area (≈140 m^2), extensive vascularization, and bypass of the liver first-pass effect. Accordingly, significant systemic bioavailabilities of a number of peptides and proteins following pulmonary administration have been reported; these include: 1-deamino-cysteine-8-D-arginine vasopressin 20% when instilled (6), increasing up to 84% when aerosolized (7) in the adult rat (absorption varied with age of the animals); leuprolide acetate 4–18% aerosolized solution or suspension in humans (8); human growth hormone ≈36% instilled in rats (9); insulin 5.6% when instilled, but 57% when aerosolized in rabbits (10); human α-interferon >56%, human calcitonin 67%, human parathyroid hormone [1-34] >40%, all when instilled in the intratracheal rat model (11); and detirelix 29% instilled in dogs (2).

Employment of animal models for pulmonary studies poses distinct problems. Instillation of rodents as well as rabbits or dogs requires general anesthesia in order to perform intubation. Aerosolization in small animals can be performed either in an aerosol tent (cage) or with a nose-only exposure module for individual animals (12) or groups of up to 60 animals (mice) (13). We have used the latter successfully to study chronic inhalation of liposomes in mice over four weeks (14). Aerosolization of large animals such as dogs again requires general anesthesia and ventilation (15), although Bennett et al. (2) report in the accompanying paper a modified procedure which requires only brief sedation and maintains spontaneous breathing at all times.

In order to avoid such complications, and to study pulmonary deposition and absorption processes on the tissue or cellular level, respectively, isolated perfused lung models have been used (16,17). Furthermore, Wall et al. (18) have recently reported an *in vitro* pulmonary epithelial system from *Xenopus* lung mounted in Ussing chambers for absorption studies.

Here, we describe the use of the unanesthetized, awake sheep as animal model for pulmonary absorption studies of detirelix. The model is particularly useful for concomitant monitoring of both drug absorption and lung function, as a measure for acute toxicity (19).

MATERIALS AND METHODS

Detirelix diacetate (M.W. 1658.3) (referred to as detirelix) was synthesized by the Institute of Organic Chemistry, Syntex Research, Palo Alto, CA. Solutions supplied by Syntex consisted of 4 mg/ml detirelix in isotonic phosphate buffered saline (PBS, 5 mM phosphate, 0.9% NaCl, pH 7.4). The dose was measured under aseptic conditions according to the weight of the individual animals as recorded the day of study. Immediately before dosing, the measured dose was diluted to a total volume of 1 ml for i.v. injection, 3 ml for intratracheal instillation, and 5 ml for aerosolization with sterile PBS. Because of the sterile handling techniques, no final sterile filtration step was performed. Rinse solutions consisted of sterile PBS, stored at 4°C before use.

Animals

Experiments were performed on a group of 8 adult fe-

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male mixed-breed sheep (61–99 kg; avg. weight 83 kg). Animals were kept indoors at all times in a climate-controlled environment on concrete floors, with 2 sheep per 14 m^2 pen, at the University of Florida Biotechnology Livestock Facility which is a NIH- and USDA-approved facility. The experimental protocol was approved by the University of Florida Animal Use Committee. Veterinary care was provided by University of Florida staff veterinarians as required. No illness or any other abnormal behavior occurred in any of the subjects during time of study.

Intravenous Injection

For assessment of absolute bioavailability, detirelix was administered slowly over 1 minute intravenously into the left or right jugular vein of 7 sheep at a dose of 30 μg/kg. Blood samples were taken from the jugular veins at time 0 (i.e. baseline taken immediately prior to dosing), 0.25, 0.5, 1, 2, 4, 8, 12, 24, 28, 36, and 48 hours post-injection. Blood samples were taken aseptically using 4 ml red-capped (non-heparinized) Vacutainers with 21 ga. double-ended needles. Whole blood was refrigerated at 4°C during clotting for ½-1 hour. Tubes were centrifuged at 2,000 rpm for 10 minutes, serum transferred to cryotubes and stored at −20°C prior to assay.

Intratracheal Instillation

Intratracheal instillation was performed by topically anesthetizing the nostrils of awake sheep with 2% Lidocaine jelly prior to intubation. As shown in Fig. 1, the animals were intubated naso-tracheally, with the distal end of the tracheal tube extending 2–3 cm below the bottom of the larynx. A dose of 80 μg/kg (corresponding to an instillation volume ranging from 1.2 ml to 2.0 ml of the 4 mg/ml detirelix solution) was injected into the trachea of 5 sheep at approximately mid-trachea through a fine catheter extending 7–8 cm beyond the end of the tracheal tube. To ensure complete delivery of the dose, the catheter was flushed with 5 ml ice-cold PBS. Blood samples were drawn at the same times and following the same procedure as described above for intravenous injection.

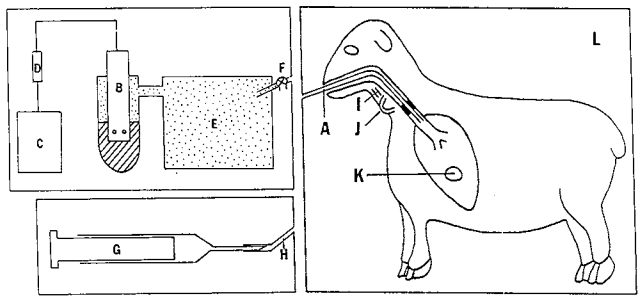

Figure 1 The unanesthetized, awake sheep is nasotracheally intubated with a cuffed endotracheal tube with a 2% lidocaine-coated tip (A). The nebulizer (B), driven by an air compressor (C) and controlled by a flow meter (D), delivers the aerosol into a reservoir (E) from which the sheep inhales via the endotracheal tube. The sheep exhales via a non-rebreather valve (F). Alternatively, intratracheal instillation is performed directly with a syringe (G) attached to a catheter (H) into the endotracheal tube (followed by a flush with saline). Blood samples for drug monitoring are taken from the jugular veins (I); blood samples for blood gas measurements can be taken from exteriorized carotid arteries (J). A pleural balloon can be surgically implanted to measure transpulmonary pressure (K). The box (L) indicates a whole body plethysmograph within which the animal can be placed during study to monitor lung function over time (J, K, and L not performed in this study).

Aerosol Inhalation

The aerosol was generated with a Puritan-Bennett model #1917 slip/stream nebulizer (60 ml reservoir; flow rate of 10–12 L/min; 50 psi; 100% O_2) into a large (40–60) liter reservoir bag from which the animals inhaled via their intratracheal tube. A non-rebreather valve (with bacterial filter on outflow) was attached to the endotracheal tube. Dead space of connecting tubing was kept to a minimum. The animals (n = 4) inhaled a nominal dose of 160 μg/kg. Corresponding volumes of 2.4 ml to 4.0 ml of the 4 mg/ml detirelix solution were aerosolized to dryness which was completed in less than 30 minutes.

Detirelix Assay

Detirelix serum concentration was determined by a specific radioimmunoassay with a lower limit of quantitation of 0.2 ng/ml (20). Reported concentrations are the average of duplicate runs and samples whose concentrations exceeded the standard curve maximum were diluted and reassayed.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed using compartmental and non-compartmental analysis as described before (19):

Non-compartmental pharmacokinetic analysis. The area under the plasma concentration-time curve (AUC) and area under the first moment curve (AUMC) was calculated using the trapezoidal rule. Mean residence time (MRT) was calculated as AUMC/AUC. The bioavailability (f) of detirelix after pulmonary administration was calculated as $[AUC_{it} \times dose_{iv}]/[AUC_{iv} \times dose_{it}] \times 100$. The mean absorption time (MAT) of detirelix after pulmonary administration was calculated as $MRT_{it} - MRT_{iv}$.

Compartmental pharmacokinetic analysis. Compartmental analysis using the nonlinear regression program RSTRIP (MicroMath, Salt Lake City, UT) was used for the estimation of the terminal half-life. Best results were obtained with a two-compartment body model for the i.v. data, whereas for the pulmonary data a one-compartment body model was satisfactory. The i.v. data was fitted to the biexponential equation

$$C_p = a \times e^{-\alpha t} + b \times e^{-\beta t}$$

From the results the half-life ($t_{1/2}$) was calculated as $\ln 2/\beta$. Pulmonary data was fitted to the biexponential equation

$$C_p = b\,(e^{-ket} - e^{-kat})$$

From the results, the half-life ($t_{1/2}$) was calculated as $\ln 2/k_e$.

RESULTS AND DISCUSSION

The resulting plasma concentration-time curves for a 30

μg/kg detirelix dose administered i.v., and a 80 μg/kg dose administered i.t., respectively, are shown in Fig. 2. An overall compilation of the pharmacokinetic data is provided in Table I.

After i.v. administration detirelix was eliminated in a biphasic fashion with a terminal $t_{1/2}$ of 7.2 ± 2.9 hours (n = 7), corresponding to a MRT of 2.7 ± 0.8 hours. The total body clearance averaged 1.17 ml/min/kg which correlated well with a clearance of 1.3 ml/min/kg and a $t_{1/2}$ of 7.1 hr following a 80 μg/kg dose in monkeys (21).

After intratracheal administration, the average $t_{1/2}$ of elimination was 9.8 ± 1.3 hours (n = 5) which was similar to the elimination kinetics of the i.v. dose. MRT was prolonged to 10.3 ± 2.0 hours and MAT was calculated to be 7.5 ± 1.8 hours. Maximum plasma levels (C_{max}) of 9.2 ng/ml were reached after 2 hours. The average bioavailability was 9.8 ± 3.9% of the dose.

While the noncompartmental parameters compare well with the parameters determined in the briefly anesthetized dog model (2), C_{max} levels were reached faster, within 2 hours compared to 6.5 hours in the dog, and the bioavailability was somewhat lower in the sheep than in the dog model (9.8% vs. 29%). However, these discrepancies may have resulted from differences in the experimental conditions rather than from intrinsic differences between the animal models. For instance, the depth of insertion of the endotracheal tube which was set directly at the bifurcation in the dog vs. extending into the mid-trachea region in the sheep may contribute to such apparent differences. Adjei and Carrigan (22) reported a large variability in the bioavailability of LHRH analogs in dogs following intratracheal instillation as a function of the distance of instillation beyond the epiglottis; bioavailabilities ranged from 4.6% to 95% when instilled 10–15 cm vs. 20–25% beyond the epiglottis. It is fair to assume that in a consenting and trained human subject the bioavailability would be at least in the 30% range and most likely higher.

Plasma levels resulting from aerosol inhalation of a 160 μg/kg dose were too low and erratic in three of the four animals to be analyzed with confidence. Only one animal generated significant plasma concentrations. It appears that the aerosol set-up with the reservoir, although thought to best reflect the clinical situation, was not suitable. This may be due to both inefficient inhalation from the reservoir by the animals as well as significant losses of aerosol due to deposition on the bag and endotracheal tubing walls. Nevertheless, the plasma curve of one animal shown in Fig. 3 is remarkably similar to the one generated from intratracheal instillation, with estimates of MRT of 19.1 hours and MAT of 14.6 hours.

Table I. Summary of Pharmacokinetic Parameters.

Route	mean ± S.D. i.v. (n = 7)	mean ± S.D. i.t. (n = 5)
AUC (ng ml^{-1} hr)	490 ± 206	112 ± 39
MRT (hr)	2.7 ± 0.8	10.3 ± 2.0
t½ (hr)	7.2 ± 2.9	9.8 ± 1.3
C_L (ml hr^{-1} kg^{-1})	70 ± 28	—
Vd_{ss} (L/kg)	0.18 ± 0.03	—
MAT (hr)	0	7.5 ± 1.8
F (%)	100	9.8 ± 3.9

Bennett et al. (2) speculate as to the prolonged absorption time and lack of difference in absorption time between instilled and inhaled doses,—which had previously been reported by Schanker et al. (23)—that the amphipathic, cationic detirelix may interact with surfactant phospholipids in the lung and could as such be absorbed and metabolized by Type II cells in the lung (24). Experiments to elucidate the pulmonary fate of detirelix are planned.

It should be noted that the plasma half-life time of up to 10 hours following instillation (and inhalation) of detirelix in the sheep is much longer than half-life times of endogenous LHRH or LHRH agonists such as nafarelin in rats and monkeys (25), or leuprolide acetate (when given as solution aerosol) in humans (8). This is likely due to an increased number of hydrophobic amino acid residues, the presence of a positively charged diethyl-homoarginine residue, and substitu-

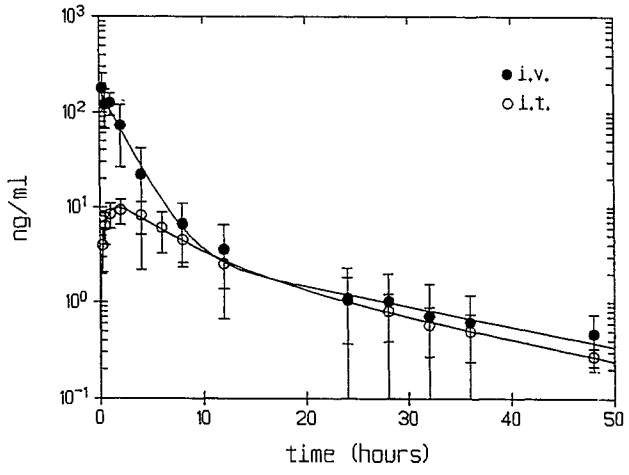

Figure 2 Plasma profiles of 30 μg/kg detirelix following intravenous (i.v.) injection and 80 μg/kg detirelix following intratracheal (i.t.) instillation in sheep. Error bars indicate mean ± S.D. (n = 7 i.v.; n = 5 i.t.).

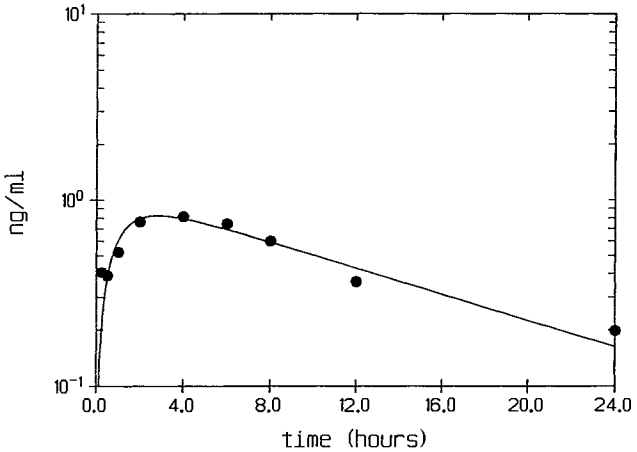

Figure 3 Plasma profile of 160 μg/kg detirelix following inhalation of an aerosol generated with a Puritan Bennett nebulizer (data of one sheep shown).

tion of the major enzymatic cleavage sites with D-amino acids, as discussed in detail by Chan *et al.* (21).

While investigators have routinely used rodents and rabbits as small, and dogs as large animal models in pulmonary absorption studies, we have employed the sheep as large animal model for such studies. The size of the animal allows frequent blood sampling, and, if desired, loops can be constructed in both exterior carotid arteries to facilitate repeated arterial blood sampling. Furthermore, as shown in Fig. 1, pleural balloons can be implanted and the animals can be placed in a whole body plethysmograph such that simultaneous monitoring of lung function and blood gases (for safety studies), as well as pharmacokinetics of instilled or inhaled drugs can be accomplished. Most advantageously, intubation, including bronchoscopy, of sheep and deposition of material (either by instillation or aerosolization) can be performed without prior general anesthesia (local anesthesia during intubation is provided). In addition, the lung volume and physiology of the sheep is comparable to the (young) human lung and, therefore, affords realistic experimental study conditions and good predictability of clinical outcomes.

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