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Bioavailability of leuprolide following intratracheal administration to beagle dogs

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Summary

Leuprolide acetate is a nonapeptide with virtually no oral bioavailability. Studies were therefore conducted to evaluate pulmonary bioavailability of this drug when administered to the lung as an aerosol. Male and female beagle dogs were administered a solution aerosol formulation of leuprolide acetate at 0, 0.5, 1, and 2 mg/day for 14 consecutive days. Results from this study demonstrated: (1) significant plasma levels of leuprolide are obtained following administration of leuprolide to the lung compared to a placebo aerosol formulation as control; (2) plasma levels of circulating leuprolide indicate a linear dose-dependent increase in pulmonary bioavailability of leuprolide from the aerosol in the dose range of 0.5–2.0 mg/dog per day; (3) basic pharmacokinetic parameters estimated from the data show no significant differences in pulmonary absorption between male and female dogs; and (4) there is a corresponding decrease in plasma gonadotropins with sequential increase in plasma leuprolide concentrations. The results further indicate that an inhalation aerosol formulation of leuprolide could be a potential alternative to parenteral administration of this drug. These findings suggest that inhalation aerosol delivery may be effective for LHRH analogs and perhaps other peptides with oral absorption limitations.

Introduction

Luteinizing hormone releasing hormone (LHRH), was discovered in 1971 (Schally et al., 1971). It is the blood-borne messenger between the hypothalamus and the anterior pituitary, which controls reproductive function. LHRH is a decapeptide hormone (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), which is synthesized and stored in the hypothalamus in neurons which project to the median eminence. LHRH is released in periodic bursts into the hypophyseal portal circulation, where it induces the release of gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) into the systemic circulation. These factors then produce a trophic and steroidogenic effect upon the gonadal tissues (Rippel et al., 1974; Johnson et al., 1976).

The discovery of LHRH led to the development of highly potent synthetic analogs in the mid

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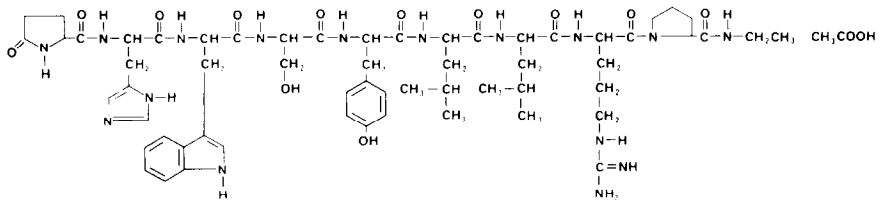

1970's (Karten and Rivier, 1986), exemplified by leuprolide (Fujino et al., 1974). Leuprolide acetate is a nonapeptide and chemically defined as: 5-Oxo-Pro-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-ethylamide acetate with the chemical structure (molecular weight: 1209.41 as free base) shown above.

Leuprolide acetate has three ionizable sites, namely the imidazolyl nitrogen of histidine ($pK_a \sim 6.0$), the phenolic hydroxyl of tyrosine ($pK_a \sim 10.0$), and the guanidine nitrogen of arginine ($pK_a \sim 13.0$). Since the guanidine nitrogen is extremely basic, this peptide, as synthesized, exists in the protonated form and is generally associated with at least one mole of acetic acid. The compound therefore exists as an acetate salt, which although an ion pair ($-NH_3^+ \cdot {}^-OOCCH_3$), has a very low octanol-water partition coefficient.

Single-dose administration of leuprolide produces a dramatic and prolonged increase in plasma LH levels (Yamazaki and Okada, 1980). In contrast, multiple dosing of leuprolide produces a paradoxical effect on the gonads as well as the reproductive processes (Rippel et al., 1975). These effects are mediated by the down-regulation of pituitary LHRH receptors, accompanied by a fall in circulating gonadotropin levels below control values (Warner et al., 1983). Thus, sex steroid production falls to castrate levels.

Leuprolide is indicated for use in treatment of prostatic cancer. Currently, it is available in an injectable form for daily subcutaneous administration, and a single dose monthly depot formulation for intramuscular administration.

Oral bioavailability of leuprolide is very low. A mixed micellar solution with bile salts and monoolein was found to have 0.05% oral bioavailability compared to an appropriate intravenously administered dose of the drug (Okada et al., 1982). The low oral absorption of this drug may be due to poor membrane permeability as well as significant enzymatic deactivation in the intestinal tract (Lee, 1986). The possibility of using the lung as a site for systemic delivery of leuprolide seemed promising. Due to the small dosage levels required for pharmacologic effects, and because the lung may possess a lower degree of enzymatic activity, an inhalation aerosol was felt to be an ideal delivery system for leuprolide.

Experimental

Animals

Twelve female and 12 male purebred beagle dogs (Marshall Research, North Rose, NY) were surgically tracheostomized and allowed to recover over a 7-day acclimation period. Following recovery from surgery, these tracheostomized dogs were fitted with intratracheal tubes during a minimum 3-day pretreatment period to ensure easy entry into the tracheal stoma. All dogs were housed in stainless-steel double-decked cages equipped with feeders and automatic waterers. Temperature and humidity conditions were monitored and maintained constant throughout the study period. Dogs were fed daily with canine diet (Ralston Purina Co., St. Louis, MO) and water was allowed ad libitum. The intratracheal tubes were removed 1 week before drug treatment was initiated.

Treatment groups and administration of dosages to dogs

The tracheostomized beagle dogs were randomly assigned within sex to four separate treatment groups. Each group was administered a daily dose of the aerosol for 14 days. A schematic representation of the animal model is presented in

Fig. 5. The study was designed to deliver drug to the bifurcation of the lung. Each aerosol vial was crimped with a 50 μl valve (Valois, DF10) and fitted to a 1.5 cm transducer device (Vortran Medical) which also served as an actuator. The head of the transducer was gently inserted into the tracheal stoma so that the tip of this actuator device was approx. 4 inches posterior to the epiglottis. In this configuration, the aerosol was directly deposited at or near the bifurcation of the lung which also resulted in minimal exhalation of aerosolized drug during expiration. The following protocol was used for drug administration:

Treatment group		Leuprolide acetate (mg/day) [a]	No. per group	
			Males	Females
Placebo	(T0)	0 (control)	3	3
Low Dose	(T1)	0.5	3	3
Middle Dose	(T2)	1	3	3
High Dose	(T3)	2	3	3

[a] Administered as a solution aerosol with each spray containing 250 μg leuprolide acetate.

To effect drug administration to the lung, the aerosol valve was activated just prior to inspiration. This ensured that inhalation of the aerosol was synchronized with inspiration in order to facilitate lung deposition of the drug. All dosages were administered within 2 min of the initial (zero time) sample.

Test material and characterization

The formulation used in the study was a solution aerosol and it contained the following ingredients:

Leuprolide acetate (Abbott Labs)	5 mg/ml
Ethyl alcohol (Quantum Chemicals, U.S.I. Div.)	34%
Decane sulfonic acid sodium salt (Sigma Chemical)	2 mg/ml
Dichlorodifluoromethane (E.I. Du Pont De Nemours)	60%
Polysorbate 80 (ICI Americas, Inc.)	2%
Purified Water, USP	4%

Material used in the control group (placebo group) was a similar formulation but it did not contain drug. Leuprolide acetate was extracted with methanol from the samples and analyzed according to a previously described HPLC assay method (Sutherland and Menon, 1987). Chemical stability of leuprolide acetate in the aerosol formulation was determined by HPLC under both accelerated storage as well as ambient temperature conditions over the period of the investigation. Physical stability data, i.e., shot weight, valve delivery, and particle size distribution, were also collected over the period of the investigation. Valve integrity was checked immediately before and after conclusion of the study.

Bioanalytical: leuprolide, testosterone and estradiol

Blood sampling was done on day 1 (first day administration of the aerosol) and also on day 14. The samples were obtained from the jugular vein at 0, 0.25, 0.5, 1.0, 2.0, 3.0, and 5.0 h after administration of the aerosol. These samples were heparinized, centrifuged and the plasma frozen until assayed for leuprolide acetate. Plasma leuprolide concentrations were determined using the radioimmunoassay technique of Yamazaki and Okada (Yamazaki and Okada, 1980). Plasma testosterone in male dogs was measured using competitive protein binding (Frick, 1974). Plasma Estradiol levels in female dogs were similarly measured using competitive protein binding (Pratt et al., 1974).

Analysis of results

Analysis of variance of the pharmacokinetic parameters for all dosage groups was performed with animal, sex, dose, group, period effects represented in the model. Differences among means were tested as significant using the Tukey-Kramer multiple comparison procedure (Lejune-Lenain et al., 1987; Veldhuis et al., 1987).

Results and Discussion

Stability of leuprolide acetate inhalation aerosol

Chemical stability data on the solution aerosol of leuprolide acetate used in the investigation over the course of the bioavailability study are summarized in Appendix I. Assay data characterizing drug residues retained on the transducer, i.e., ex-

valve delivery results after day 1 and day 14 of dosing, are also summarized in Appendix I. These data show that approx. 15% (range 12.6–18.2%) and approx. 12% (range 11.1–13.6%) of leuprolide acetate was retained on the delivery device after day 1 dosing and day 14 dosing, respectively. Functional performance parameters on the aerosol, i.e., shot weight, leakproofness, unit spray content, and particle size distribution were also monitored and found to be satisfactory. The experimental method to support dose uniformity and particle size distribution in leuprolide aerosols together with interpretation of the data are described elsewhere (Adjei and Garren, 1990) and are therefore not reported here.

Plasma concentrations of leuprolide acetate

Plasma concentration time profiles of leuprolide on days 1 and 14 for the three dosage groups are summarized in Figs 1 and 2 for the male and female dogs, respectively. Pooled data for both sex groups are shown in Fig. 3. Geometric means from fitted regression lines of the data, $AUC_{(0-5\ h)}$ on dose for days 1 and 14 are shown in Fig. 4 for

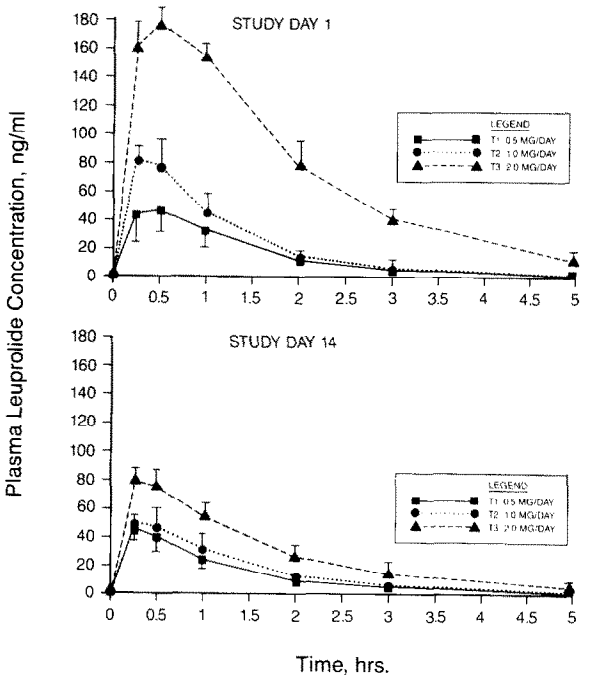

Fig. 2. Plasma level profile of leuprolide following intratracheal administration to female beagle dogs.

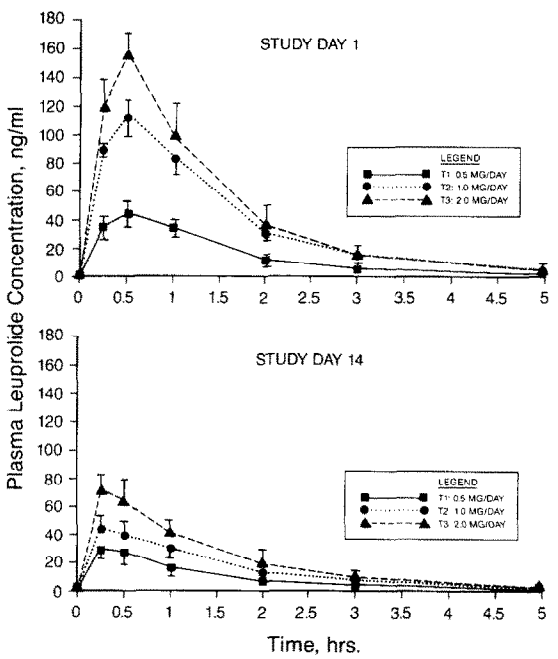

Fig. 1. Plasma level profile of leuprolide following intratracheal administration to male beagle dogs.

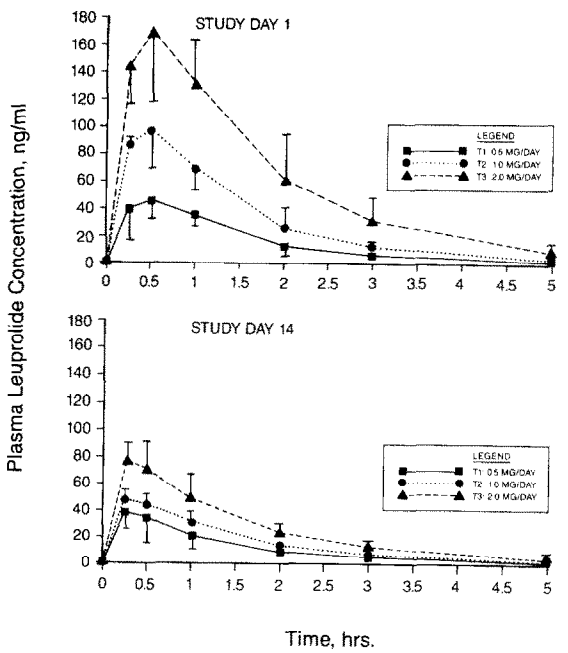

Fig. 3. Plasma level profiles of leuprolide following intratracheal administration to beagle dogs.

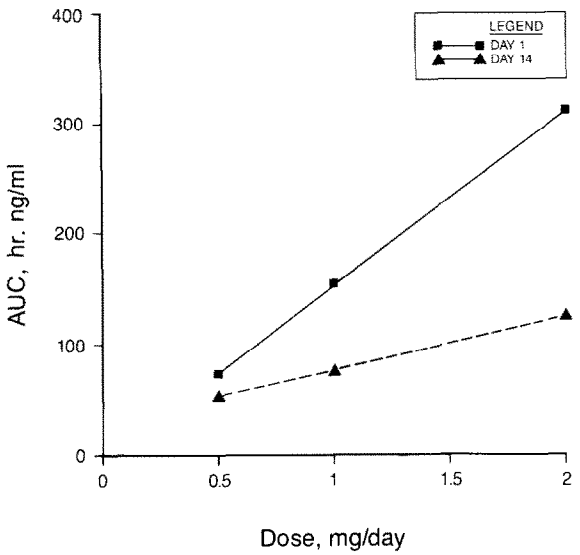

Fig. 4. Pulmonary bioavailability of leuprolide regression of AUC on dose in beagle dogs.

the respective dosage groups. This graph clearly shows a linear dose response relationship with pulmonary bioavailability of leuprolide within the dose range of 0.5–2.0 mg per dog. In all dosage groups, absorption of leuprolide from the lung is rapid with mean peak plasma levels occurring within approx. 30 min after dosing.

Plasma concentrations of leuprolide and mean AUC values calculated for the 1.0 and 2.0 mg dosage groups showed bioavailabilities on day 14 to be approximately half of those obtained on day 1 (Tables 1 and 2). A 20% decrease in the AUC value was observed for the 0.5 mg dose group on day 14 compared to day 1. This observation was unexpected and the reason for the difference is not clear. There may be two possible explanations as to the reduction in plasma AUC following chronic administration of the drug via the lung. First, decrease in plasma levels with multiple dosing may be related to a tolerance build-up and/or increased enzymatic activity of the lung following chronic administration. Although no gross histologic and pathologic changes were observed at the end of the 14 day study period, mild microscopic and inflammatory reactions of the lung tissue were noted in post mortem examinations. Similar observations were noted with the placebo control group although for all the dogs laboratory data (vital signs and blood chemistry) before and after the study period showed no significant deviations from normal results. Second, the apparent decrease in AUC may be model dependent. Deep and slow inspiration facilitates pulmonary deposition of inhaled particles (Agnew, 1984; Padfield, 1987). Since dosimetric requirements (shot weight, leakproofness, unit spray content, and particle size distribution) were stable through the study period, microscopic changes in the lung and/or respiratory pattern differences during drug administra-

TABLE 1

Summary statistics on plasma data after 1 day of pulmonary delivery to the lung of male and female beagle dogs

Treatment group	Dog no.	CMAX (ng/ml)	TMAX (h)	AUC (h ng ml^{-1})
Leuprolide				
0.5 mg/day	1001	36.2	0.50	54.2
	1002	24.7	0.50	38.1
	1003	44.3	0.50	77.6
	1004	42.1	0.50	66.7
	1005	51.1	0.50	86.0
	1006	84.8	0.25	116.5
	mean	47.2	0.46	73.2
	SD	20.4	0.10	27.2
1.0 mg/day	2001	118.9	0.50	179.2
	2002	85.0	0.25	85.9
	2003	102.4	0.50	171.7
	2005	118.4	0.50	206.6
	2006	91.0	0.50	127.8
	mean	103.1	0.45	154.2
	SD	15.5	0.11	47.5
2.0 mg/day	2004	181.0	1.00	386.9
	3001	225.4	0.50	306.0
	3002	178.1	0.25	343.1
	3003	138.7	0.50	177.1
	3004	258.4	0.50	549.1
	3005	104.0	0.50	195.5
	3006	125.8	0.25	220.9
	mean	173.1	0.50	311.2
	SD	55.2	0.25	130.9
Pairwise differences		1 2 3 [a]	NS [b]	1 2 3

[a] Multiple comparison results: 1, 2, and 3 denote treatment groups T1, T2, and T3, respectively. Groups not underscored by the same line were significantly different ($p <= 0.05$).

[b] NS, no significant differences among treatment groups ($p > 0.05$).

TABLE 2

Summary statistics on plasma data after 14 days chronic administration of leuprolide to the lung of male and female beagle dogs

Treatment group	Dog no.	CMAX (ng/ml)	TMAX (h)	AUC (h ng ml^{-1})
Leuprolide				
0.5 mg/day	1001	20.1	0.25	26.3
	1002	67.9	0.25	95.0
	1003	40.0	0.50	62.8
	1004	48.1	0.25	64.3
	1005	30.3	0.25	42.5
	1006	21.6	0.25	26.9
	mean	38.0	0.29	53.0
	SD	18.2	0.10	26.4
1.0 mg/day	2001	43.1	0.25	67.0
	2002	34.2	0.50	54.3
	2003	40.1	0.25	62.1
	2004	70.8	0.25	93.4
	2005	49.7	0.25	95.5
	2006	50.2	0.50	82.9
	mean	48.0	0.33	75.9
	SD	12.7	0.13	17.2
2.0 mg/day	3001	77.0	0.25	128.6
	3002	101.4	0.50	193.0
	3003	74.2	0.25	98.7
	3004	64.1	0.50	118.9
	3005	63.8	0.25	100.7
	3006	79.7	0.25	111.2
	mean	76.7	0.33	125.2
	SD	13.8	0.13	35.1
Pairwise differences		1 2 3 [a]	NS [b]	1 2 3

[a] Multiple comparison results: 1, 2, and 3 denote treatment groups T1, T2, and T3, respectively. Groups not underscored by the same line were significantly different ($p < = 0.05$).
[b] NS, no significant differences among treatment groups ($p > 0.05$).

tion on day 1 vs day 14 may have contributed to the disparity in these results.

Of the two reasons presented to explain differences in pulmonary bioavailability of leuprolide acetate between day 1 and day 14, the effect of changes in respiratory patterns as evidenced by minor lung irritation reactions at the end of the 14 day study period may be the most plausible. Dogs were not anesthetized prior to initiation of the study. The technique for administering the aerosol (Fig. 5) required synchronization of inspiration with activation of the aerosol. It further required a 5–10 s breath-hold before exhalation. Controlled

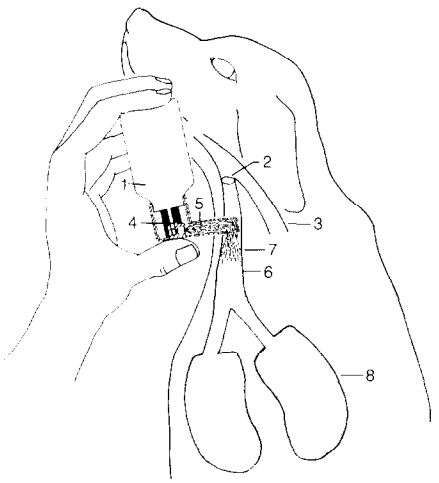

Fig. 5. Schematic diagram of dog model for intratracheal administration of leuprolide aerosol formulations.

exhalation and any changes in respiration rates immediately after drug administration may be potential factors that would affect lung deposition and retention of pharmaceutical aerosols. These factors could not be controlled in the dog model used. This is a disadvantage in contrast with other models requiring the use of gas masks from which the administered aerosol may be breathed by

TABLE 3

Pulmonary delivery of leuprolide in male beagle dogs: summary statistics on plasma data after first day of dosing

Treatment group		CMAX (ng/ml)	TMAX (h)	AUC (h ng ml^{-1})
Leuprolide				
0.5 mg/day	mean	43.9	0.50	72.6
	SD	7.5	0.00	16.5
1.0 mg/day	mean	113.2	0.50	185.8
	SD	9.4	0.00	18.4
2.0 mg/day	mean	156.0	0.50	226.2
	SD	62.5	0.00	69.7
Leuprolide 1 mg/dog iv bolus dose	mean	519.0	–	405.1
	SD	118.0		58.4
Pairwise differences		1 2 3 [a]	– [b]	1 2 3

[a] Multiple comparison results: 1, 2, and 3 denote treatment groups T1, T2, and T3, respectively. Groups not underscored by the same line were significantly different ($p < = 0.05$).
[b] –, no statistical analysis done.

trained dogs. However, the current model has a clear advantage in that precise quantities of drug could be administered to the respiratory tree in order to enable estimation of both absolute as well as apparent bioavailabilities of the aerosol.

To estimate absolute bioavailability of leuprolide from the lungs following administration of the aerosol, the area under the plasma concentration vs time curve up to 5 h (AUC) for a single 1 mg intravenously administered dose of leuprolide acetate was obtained for 12 male beagle dogs. These data are summarized in Table 3. Examination of the AUC values obtained for all dogs on both days 1 and 14 relative to the mean AUC of the intravenous control group indicates 36–38% absolute bioavailability (uncorrected for drug retained on the transducer) on day 1. The absolute bioavailability of leuprolide acetate on day 14 was 15–26%. The higher variability in the bioavailability values estimated for day 14 are unexpected and cannot be explained by drug residues retained on the delivery device as deduced from the data in Appendix 1. This variability, as explained earlier, could have resulted from microscopic changes in the environment within the lung following chronic administration of the aerosol.

Summaries of pharmacokinetic data, namely, CMAX, TMAX, and AUC for leuprolide following inhalation administration of the three respective dosage groups are summarized in Tables 3 (day 1) and 4 (day 14) for the males. Corresponding data for the females are summarized in Tables 5 (day 1) and 6 (day 14). Tables 1 (day 1) and 2 (day 14) contain mean data as well as individual pharmacokinetic data for all dogs used in the study. Analysis of variance showed no significant treatment-by-sex interaction nor significant sex effect for the three variables analyzed.

(1) CMAX: On day 1 the high dose males and the high dose females had significantly greater

TABLE 4

Summary statistics on plasma data after 14 days chronic administration to the lung of male beagle dogs

Treatment group		CMAX (ng/ml)	TMAX (h)	AUC (h ng ml^{-1})
Leuprolide				
0.5 mg/day	mean	30.1	0.33	43.9
	SD	9.9	0.14	18.3
1.0 mg/day	mean	44.3	0.25	74.9
	SD	4.9	0.00	18.0
2.0 mg/day	mean	71.7	0.25	109.3
	SD	7.0	0.00	16.7
Pairwise differences		1 2 3 [a]	NS [b]	1 2 3

[a] Multiple comparison results: 1, 2, and 3 denote treatment groups T1, T2 and T3, respectively. Groups not underscored by the same line were significantly different ($p < = 0.05$).

[b] NS, no significant differences among treatment groups ($p > 0.05$).

TABLE 5

Pulmonary delivery of leuprolide in female beagle dogs: summary statistics on plasma data after first day of dosing

Treatment group		CMAX (ng/ml)	TMAX (h)	AUC (h ng ml^{-1})
Leuprolide				
0.5 mg/day	mean	50.5	0.42	73.8
	SD	30.9	0.14	39.7
1.0 mg/day	mean	88.00	0.38	106.8
	SD	4.24	0.18	29.6
2.0 mg/day	mean	185.8	0.50	375.0
	SD	54.6	0.35	135.6
Pairwise differences		1 2 3 [a]	NS [b]	1 2 3

[a] Multiple comparison results: 1, 2, and 3 denote treatment groups T1, T2, and T3, respectively. Groups not underscored by the same line were significantly different ($p < = 0.05$).

[b] NS, no significant differences among treatment groups ($p > 0.05$).

TABLE 6

Summary statistics on plasma data after 14 days chronic administration of leuprolide to the lung of female beagle dogs

Treatment group	Dog no.	CMAX (ng/ml)	TMAX (h)	AUC (h ng ml^{-1})
Leuprolide				
0.5 mg/day	mean	45.9	0.25	62.1
	SD	23.2	0.00	34.1
1.0 mg/day	mean	51.7	0.42	76.9
	SD	18.3	0.14	20.2
2.0 mg/day	mean	81.7	0.42	141.0
	SD	18.7	0.14	45.2
Pairwise differences		NS	NS	NS

NS, no significant differences among treatment groups ($p > 0.05$).

mean values than the corresponding low dose male and low dose females. When the males and females were combined, the mean of the high dose dogs was significantly greater than those of the low and middle dose dogs, indicating significant peak plasma levels of leuprolide at 2 mg/day compared to 0.5 and 1 mg/day, respectively.

On day 14 the high dose males had a significantly greater mean than the low and middle dose males. There were no significant differences among the females. The analysis combining males and females showed the mean of the high dose dogs to be significantly greater than those of the low and middle dose dogs indicating significant pulmonary absorption of leuprolide at 2 mg/day compared to 0.5 and 1 mg/day, respectively.

TABLE 7

Summary statistics on plasma testosterone (ng/dl) levels following chronic administration of leuprolide aerosol to the lung of male beagle dogs

Treatment group	Dog no.	Day 1	Day 14
Control	0001	337.0	121.0
	0003	369.0	125.0
	0005	76.9	113.0
	mean	261.0	119.7
	SD	160.2	6.1
Leuprolide			
0.5 mg/day	1001	237.0	20.9
	1003	NS [a]	NS
	1005	61.3	35.1
	mean	149.2	28.0
	SD	124.2	10.0
1.0 mg/day	2001	90.4	13.8
	2003	147.0	14.4
	2005	764.0	30.2
	mean	333.8	19.5
	SD	373.6	9.3
2.0 mg/day	3001	244.0	12.5
	3003	116.0	48.4
	3005	15.1	11.6
	mean	125.0	24.2
	SD	114.7	21.0
Pairwise differences		NS [b]	2 3 1 0 [c]

[a] NS, quantity not sufficient.

[b] NS, no significant differences among treatment groups ($p >= 0.05$).

[c] Multiple comparison results: 0, 1, 2 and 3 denote treatment groups T0, T1, T2 and T3, respectively. Groups not underscored by the same line were significantly different ($p <= 0.05$).

(2) TMAX: This variable yielded no statistically significant treatment differences.

(3) AUC: The AUC data were not transformed for this pairwise comparison. On day 1 the middle and high dose males had mean AUC values that were significantly greater than the mean AUC value for the low dose males. The high dose females also had a mean AUC value that was significantly greater than those of the low dose and middle dose females. When the males and females were analyzed together, the mean AUC of all the high dose dogs was significantly greater than the mean AUC values of the low and middle dose dogs indicating significantly greater pulmonary absorption of leuprolide at 2 mg/day compared to 0.5 and 1 mg/day, respectively.

On day 14, the mean AUC of the high dose males was significantly greater than the mean AUC of the corresponding low dose males. However, similar comparative analysis did not show any significant differences among the females. Analysis of variance of pooled data that combined both gender AUC data showed mean AUC data for pulmonary absorption of leuprolide for the high dose dogs was significantly greater than the respective mean AUC values for the low and middle dose dogs. This finding again suggests that there is significantly greater pulmonary absorption of leuprolide at 2 mg/day compared to 0.5 and 1 mg/day, respectively.

The regression analysis of the AUC data showed that on days 1 and 14 the regression coefficient of AUC vs dose was significantly different from zero and that the data fitted a simple linear regression model, i.e., the test for lack of fit was not statistically significant. The intercept, the slope, and the standard error of the slope of each regression line were:

	Intercept	Slope	Std. Err. Slope	Corr. Coeff.
Day 1	−5.30	158.4	1.64	0.9999
Day 14	28.35	48.3	0.87	0.9998

Comparison of the two regression lines showed that the slopes were significantly different from each other again suggesting that the level of the response (AUC) on day 1 was significantly higher than the level on day 14.

Plasma concentrations of testosterone

Plasma testosterone levels determined in male dogs on days 1 and 14 of the study are given in Table 7. Statistically significant decreases of plasma testosterone levels were observed between all male treatment groups and controls on day 14. The comparison of testosterone levels measured on days 1 and 14 showed a decrease of greater than 80% in 5 of 8 treated dogs. The remaining 3 treated dogs that showed much smaller decreases in testosterone levels after 14 days of drug administration also had lower initial plasma testosterone levels. This may account for the noted difference between this group of dogs compared to the other five dogs. All treated dogs had plasma levels of testosterone on day 14 that were near castrate, i.e., less than 50 ng/dl. This finding is consistent with the known pharmacological activity of leuprolide after parenteral administration (Warner et al., 1983). Although two of three control males showed a decrease in plasma testosterone to approx. 120 ng/dl, this effect may have been associated with the circadian rhythm of testosterone secretion (Lejume-Lenain et al., 1987; Veldhuis et al., 1987). Summary statistics of plasma testosterone data following pulmonary administration of leuprolide in the three dosage groups are summarized at the bottom of Table 7. These data indicate that on day 14 all dose groups had significantly lower plasma testosterone concentrations than the control group.

Plasma concentrations of estradiol

Plasma estradiol levels were determined in female dogs and found to be at the limit of the assay possibly due to the fact that all of the female dogs may have been at their pre-estrous state. Overall, there was no trend in plasma estradiol levels over the period of this investigation. Hence, the data showed no definite effect of pulmonary administration of leuprolide on pharmacologic effects as would be expected by demonstration of significant decreases in plasma estradiol levels.

Conclusions

Inhalation delivery of leuprolide in beagle dogs resulted in significant plasma levels of the drug. Bioavailability of leuprolide aerosol following lung administration resulted in dose-dependent increases in the area under the plasma-time course curve for leuprolide acetate at dosage levels of 0.5–2.0 mg/dog. Bioavailability of leuprolide on day 1 was approximately double that on day 14 and the data suggested a possibility of up to 40% absorption of the drug from the lung following administration as an inhalation aerosol. The results also showed that plasma testosterone levels were reduced to castrate levels as a result of drug administration by the inhalation route. A significant effect on plasma estradiol levels could not be shown over the 2-week treatment period. This was due to low initial baseline levels of estradiol. These findings suggest that peptides, like leuprolide acetate, may be effectively delivered systemically using inhalation aerosols.

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Appendix I

Chemical stability as a function of time and temperature leuprolide acetate aerosol (5 mg/ml)

Time (months)	Storage (condition)	Leuprolide assay by HPLC (% LC)	Physical appearance (color and clarity)
00D	Initial	100.6	Meets requirements
01M	50 °C	90.8	Meets requirements
02M	50 °C	93.4	Meets requirements
03M	50 °C	93.6	Meets requirements
01M	40 °C	87.8 *	Meets requirements
02M	40 °C	93.0	Meets requirements
03M	40 °C	94.4	Meets requirements
01M	30 °C	90.6	Meets requirements
02M	30 °C	104.0	Meets requirements
03M	30 °C	102.0	Meets requirements
01M	25 °C	102.0	Meets requirements
02M	25 °C	103.6	Meets requirements
03M	25 °C	95.8	Meets requirements
03M	05 °C	96.6	Meets requirements
06M	05 °C	98.0	Meets requirements

* Assay result outside of specification limits of 90–120% of label claim (LC).

Summary of ex-valve delivery data for leuprolide acetate aerosol (5 mg/ml) after storage under ambient conditions

Drug treatment group	Leuprolide assay by HPLC (% of label claim)	
	% drug per dose	% drug retained on device
Study day 1		
0.5 mg/day	97.4%	16.5%
1.0 mg/day	99.1%	12.6%
2.0 mg/day	97.9%	18.2%
Study day 14		
0.5 mg/day	98.1%	13.6%
1.0 mg/day	101.4%	12.2%
2.0 mg/day	99.7%	11.1%

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