Pharmacokinetics of Once-a-Month Injectable Microspheres of Leuprolide Acetate[1]

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The pharmacokinetic parameters of leuprolide acetate, a potent analogue of LH-RH, were determined in rats and dogs after i.v. and s.c. dosing with leuprolide solution. The effective human dose of once-a-month injectable microspheres of leuprolide was estimated to be about 3.2 to 8.1 mg analogue/month using these parameters. After microsphere injection at three different doses in rat serum leuprolide concentrations were sustained for over 4 weeks, and the AUCs and mean serum levels were linearly correlated with the dose. The serum levels and urinary excretion of the analogue in rats after repeated s.c. injection of the microspheres every 4 weeks exhibited similar profiles after each injection; no changes of the absorption and excretion of the analogue after the repeated injection could be demonstrated. The serum levels of the analogue metabolite (M-I) were 21% of the intact form 3 hr after injection of the microspheres but very low at the steady state after 1 to 4 weeks.

KEY WORDS: leuprolide (leuprorelin); once-a-month injectable microspheres; pharmacokinetics; urinary excretion; metabolite.

INTRODUCTION

We have recently developed once-a-month injectable microspheres of leuprolide acetate using copoly(DL-lactic/glycolic acid) prepared by a novel in-water drying method (1–3). Leuprolide [leuprorelin, D-Leu^6-(des-Gly^{10}-NH_2)-LH-RH ethylamide], a potent analogue of luteinizing hormone-releasing hormone (LH-RH), is useful upon chronic administration for treating hormone-dependent prostate and mammary tumors (4,5) and endometriosis (6). Our previous studies (6–9) demonstrated that a single injection of the microspheres provided the sustained serum levels of leuprolide and persistent inhibition of gonadotropin release, steroidogenesis, and weight gain of the reproductive organs for over 1 month; sufficient therapeutic efficacy in the treatment of prostate cancer and endometriosis was therefore expected. Use of this long-acting depot formulation not only eliminates the inconvenience of conventional daily injection of the analogue solution by patients, but also would increase patient compliance and assure greater therapeutic efficacy by providing constant agonist concentrations at the target organ receptors.

In this experiment, pharmacokinetic parameters of leuprolide in rats and dogs after injection of the solution were determined by radioimmunoassay (RIA) in order to estimate the microsphere dose for humans. The relationship of the dose to the serum levels of the analogue after a single injection of the microspheres and the serum concentrations and urinary excretion following the repeated injection every 4 weeks were also determined. Additionally, the serum levels of the analogue metabolite (M-I, Tyr-D-Leu-Leu-Arg-Pro-NH-C_2H_5) were determined using a new analytical method (10).

MATERIALS AND METHODS

Animals and Materials

Male and female Sprague–Dawley rats and male Beagle dogs purchased from Clea Japan, Inc. (Tokyo), were used. Leuprolide acetate and the metabolite (M-I) were synthesized in the research laboratories of our company. Copoly(DL-lactic/glycolic acid) (PLGA) was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo); PLGA (76.7/23.3)-12,100 for Lot M07 and PLGA (76.0/24.0)-14,000 for Lot T002. The numbers in parentheses represent the molar ratio of lactic/glycolic acid, followed by the weight-average molecular weight. The mircospheres of leuprolide were prepared by the in-water drying method (9).

Radioimmunoassay of Leuprolide and M-I

Leuprolide in serum and urine was determined in duplicate by a double-antibody RIA system (6). The metabolite (M-I) was assayed by Ueno's method (10) using a RIA system after separation of the intact form and metabolite by high-performance liquid chromatography. The detection limits of the assays were 63 and 25 pg/ml for serum and urine leuprolide acetate, respectively, and 50 pg/ml for serum M-I. The coefficient of variance (50–1000 pg/tube) was <11.2% intraassay and <19.2% inter-assay.

Pharmacokinetics in Rats and Dogs

Leuprolide acetate dissolved in saline solution was injected i.v. or s.c. into male rats (8 weeks of age) and dogs (1.5 years of age) at a dose of 100 μg/kg. Blood was serially withdrawn from the tail (rat) or foreleg (dog) vein and kept under ice. Serum was separated after clotting at below 4°C and was stored at below −40°C until assay of the leuprolide concentrations. Pharmacokinetic parameters were calculated using NONLIN by an open two-compartment model as previously described (11).

The microspheres (Lot M07) were injected s.c. into the back of male rats (10 weeks of age) at doses of 1.35, 3.38, and 6.75 mg analogue/rat, corresponding to doses of 3, 7.5, and 15 mg/kg (average body weight of 450 g), respectively. Blood was serially collected from the tail vein for 5 weeks.

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Table I. Serum Levels of Leuprolide Acetate in Rats and Dogs After i.v. and s.c. Injection of the Solution[a]

Time	Rat (ng/ml)		Dog (ng/ml)	
	i.v.	s.c.	i.v.	s.c.
5 min	142.3 (5.7)	—	363.9 (18.1)	—
10 min	123.2 (4.8)	—	288.3 (15.8)	—
15 min	—	46.4 (2.7)	—	114.6 (7.0)
20 min	95.4 (1.4)	—	234.9 (4.6)	—
30 min	77.2 (2.9)	44.1 (1.7)	183.7 (6.4)	150.2 (14.8)
45 min	53.6 (2.1)	38.3 (1.8)	144.6 (7.3)	129.9 (15.4)
1 hr	46.3 (4.2)	40.2 (3.2)	114.2 (6.5)	138.6 (8.4)
1.5 hr	21.3 (1.9)	—	81.7 (6.6)	122.2 (2.7)
2 hr	12.3 (1.3)	24.0 (2.6)	57.2 (2.2)	81.3 (4.7)
3 hr	6.82 (1.2)	14.4 (1.6)	30.4 (1.3)	54.7 (2.2)
4 hr	1.40 (0.6)	6.67 (0.4)	17.7 (0.7)	32.6 (1.4)
6 hr	0.06 (0.1)	0.62 (0.3)	7.65 (1.2)	13.8 (1.5)

[a] Leuprolide acetate was injected as the saline solution at a dose of 100 μg/kg. Each value represents the mean (SE) of five rats and dogs.

Repeated Injection of the Microspheres

To determine the serum levels and urinary excretion of leuprolide, the microspheres (Lot T002) were chronically injected s.c. (one injection every 4 weeks, a total of three times) into male (10 weeks of age) and female (11 weeks of age) rats. The microspheres were injected at a dose of approximately 3 mg analogue/kg. Doses of the microspheres for the three injections were 1.35 (first), 1.65 (second), and 1.65 (third) mg analogue/rat for males and 0.9 (first), 1.35 (second), and 1.35 (third) mg/rat for females. Blood was serially collected from the tail vein after each injection and serum leuprolide was determined. Mean body weight of rats 3 weeks after injection was 444 (first), 484 (second), and 514 (third) g for males and 332 (first), 363 (second), and 390 (third) g for females. The individual serum levels were corrected using the measured body weight of each rat and adjusted to the value at the dose of 3 mg/kg. The difference between each AUC was analyzed statistically using ANOVA. Twenty-four-hour urine samples were collected on days 0, 2, 7, 14, 21, and 28 after each injection as well as on days 42 and 56 after the last injection. The urine was collected at room temperature for 24 hr, during which no decay of the drug was ascertained, diluted to 50 ml by adding distilled water, and frozen at below −40°C until the day of the assay.

Serum M-I After Injection of the Microspheres

The microspheres (Lot T002) were injected s.c. into the back of male rats (10 weeks of age) at a dose of 1.35 mg of leuprolide acetate. Blood was collected serially from the abdominal aorta under ether anesthesia and the serum concentrations of intact leuprolide and M-I were assayed.

RESULTS AND DISCUSSION

Pharmacokinetics in Rats and Dogs

The serum levels of leuprolide acetate in male rats and dogs after i.v. and s.c. injection of the drug solution are shown in Table I. The pharmacokinetic parameters are shown in Table II together with those calculated using the human data reported by Sennello *et al.* (12). The disappear-

Table II. Pharmacokinetic Parameters of Leuprolide Acetate in Rats and Dogs After i.v. and s.c. Injection of the Solution and After s.c. Injection of the Depot Formulation[a]

	Rat	Dog	Human[b]
i.v. injection			
A (ng/ml)	83.6 (31.3)	293.6 (53.8)	99.3 (13.9)
B (ng/ml)	110.4 (23.6)	186.2 (26.1)	22.3 (3.80)
α (hr^{-1})	7.62 (3.65)	5.50 (2.36)	2.68 (0.24)
β (hr^{-1})	1.04 (0.10)	0.58 (0.06)	0.24 (0.02)
k_{12} (hr^{-1})	2.52 (1.97)	2.14 (1.41)	1.29 (0.15)
k_{21} (hr^{-1})	4.55 (1.65)	2.45 (0.84)	0.68 (0.05)
k_e (hr^{-1})	1.59 (0.21)	1.23 (0.18)	0.96 (0.09)
V_c (L/kg)	0.56 (0.07)	0.22 (0.02)	9.18 (1.46)[c]
$T_{1/2 \cdot \alpha}$ (min)	5.5	7.56	15.5
$T_{1/2 \cdot \beta}$ (hr)	0.67	1.19	2.89
s.c. injection			
Cl_{tot} (L/day/kg)	22.5	5.71	2.89
Ratio	7.8	2.0	1.0
C_{ss} at 75 μg/kg/day (ng/ml)	3.34	13.1	26.0
Release rate attaining 1 ng/ml of C_{ss} (μg/kg/day)	22.5	5.71	2.89

[a] Serum level of leuprolide acetate (C_t) at time t, $C_t = Ae^{-\alpha t} + Be^{-\beta t}$; transfer rate constant, k_{12} (from central to tissue), k_{21} (from tissue to central); elimination rate constant, k_e; distribution volume of central, V_c. Each value represents the mean (SE) of five animals or six humans. Total-body clearance, Cl_{tot}; steady-state serum level, C_{ss}.

[b] Calculated using the data reported by Sennello *et al.* (12).

[c] L.

ance of leuprolide from the serum was very rapid in rats but slower in dogs and humans. The total-body clearance (Cl_{tot} = dose/AUC) after s.c. injection of the analogue solution was rat > dog > human (7.8:2:1). The steady-state serum drug concentrations (C_{ss}) after s.c. constant infusion is obtained by dividing the infusion rate by Cl_{tot}. In the case of administration of leuprolide acetate by the microspheres, the dose of 100 μg/kg/day at steady state for 30 days corresponds to approximately 75 μg/kg/day after subtracting the dose loss, such as the initial burst (about 20%) and the amount remaining at the injection site 4 weeks after injection (about 5%), from the dose (6). The required release rate to attain an effective plateau serum level is determined by multiplying the required serum level by Cl_{tot}. These calculated values are shown in Table II (s.c. injection).

In our previous study evaluating steroidogenesis and weight gain of the reproductive organs in rats after constant s.c. infusion using Alzet minipumps, the minimal effective serum level was estimated to be about 0.4 to 1 ng/ml (7). The dose required to attain the same effective serum level in humans is 1/7.8 of that required in the rat, which is directly related to Cl_{tot}. This release rate (dose) is 1.16 to 2.89 μg/kg/day for 0.4 to 1 ng/ml, corresponding to a microsphere dose of 3.2 to 8.1 mg leuprolide acetate/month/man with a body weight of 70 kg after correction for the dose loss (25%) (1 ng/ml: 2.89 × 30 × 70/0.75 = 8.1). This estimated effective dose of 8 mg/month is very close to the therapeutic clinical dose of 7.5 mg/month in the United States. The calculated C_{ss} in dogs following constant infusion at a dose of 75 μg/kg/day is 13.1 ng/ml (Table II), and this correlates well with the experimental value after s.c. injection of the microspheres in our previous study (8).

In clinical studies in patients with advanced prostatic cancer reported by Sharifi *et al.* (13), sustained serum concentrations, about 0.8 ng/ml, of leuprolide acetate and persistent suppressions of gonadotropin and testosterone releases were achieved after once-a-month repeated injection of the microspheres at a dose of 7.5 mg leuprolide acetate/month. As a result, by using the microspheres, the required dose is reduced to one-fourth of that required when using the conventional daily parenteral formulation of the leuprolide solution (1 mg/day).

The serum leuprolide concentrations in rats after injection of the microspheres at three different doses were well

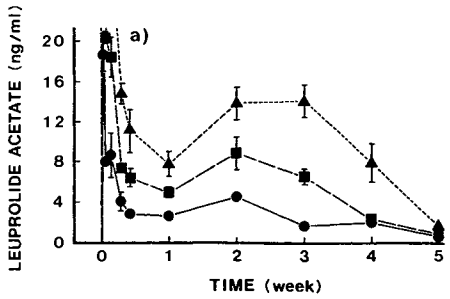

Fig. 1. Serum levels of leuprolide acetate in rats after s.c. injection of the microspheres at three different doses (●, 3 mg/kg; ■, 7.5 mg/kg; ▲, 15 mg/kg) of leuprolide acetate. Each point represents the mean ± SE of five or six rats. (a) 7.5 mg/kg—$C_{3\ hr}$ = 50.3 ± 6.3 ng/ml; 15 mg/kg—$C_{3\ hr}$ = 139.4 ± 21.8 ng/ml, $C_{6\ hr}$ = 44.5 ± 7.5 ng/ml, $C_{1\ d}$ = 27.8 ± 7.8 ng/ml (m = 2).

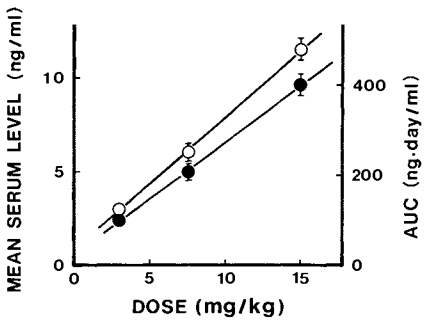

Fig. 2. AUC for 0 to 5 weeks (●) and mean leuprolide acetate serum levels 2 days to 4 weeks (○) after s.c. injection of the microspheres at three different doses in rats. These data are from the same experiment as that in Fig. 1. Each point represents the mean ± SE of five or six rats.

sustained for over 4 weeks following an initial burst (Fig. 1). These blood level curves were similar in shape and proportional to the dose. The mean serum level 2 days to 3 weeks after a dose of 3 mg/kg was 3.1 ng/ml, and this is similar to the calculated plateau level (C_{ss}) shown in Table II. AUCs of the serum levels for 5 weeks and the mean serum levels 2 days to 4 weeks after injection are shown in Fig. 2. Both parameters are correlated linearly with the dose. These results indicate that linear pharmacokinetic profiles in the absorption, distribution, metabolism, and excretion could be observed at a dose of 3 to 15 mg/kg.

Repeated Injection of the Microspheres

The serum concentration and urinary excretion of leuprolide in male and female rats after repeated s.c. injection of the microspheres (one injection every 4 weeks) were determined. As shown in Fig. 3, the serum concentrations for

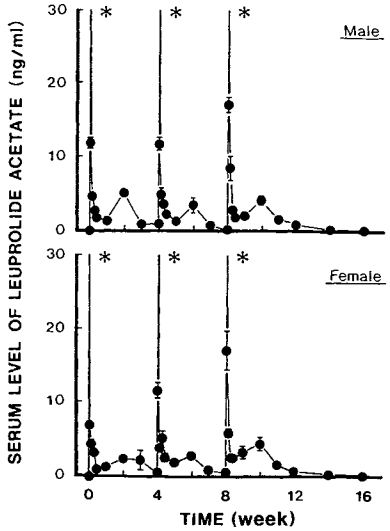

Fig. 3. Serum levels of leuprolide acetate in rats after repeated s.c. injection of the microspheres, one injection every 4 weeks. The dose was 3 mg/kg of leuprolide acetate. Each value represents the mean ± SE of six rats. (*), serum levels 3 hr after injection (ng/ml): male—62.6 ± 4.0 (first), 54.7 ± 3.3 (second), 76.0 ± 6.6 (third); female—56.9 ± 5.5 (first), 67.8 ± 10.1 (second), 62.1 ± 5.3 (third).

Table III. AUC (0–4 weeks) of Serum Levels of Leuprolide Acetate in Rats After Repeated s.c. Injection of the Microspheres[a]

Injection	Male (ng · day/ml)	Female (ng · day/ml)
1st	75.6 ± 6.0	59.6 ± 9.2
2nd	54.3 ± 7.9 (ns)[b]	57.9 ± 4.5 (ns)
3rd	76.6 ± 6.4 (ns)	83.1 ± 10.6 (ns)

[a] These data are from the same experiment as that in Fig. 3. Each value represents the mean ± SE of six rats.
[b] Not significantly different from the first injection by ANOVA ($P > 0.05$).

Table IV. Serum Levels of the Intact Form and Metabolite (M-I) of Leuprolide Acetate in Rats After s.c. Injection of the Microspheres[a]

Time	Intact (ng/ml)	M-I (ng/ml)	%[b]
3 hr	49.5 ± 3.9	10.5 ± 3.5	21.2
1 week	2.65 ± 0.51	0.09 ± 0.02	3.4
2 weeks	4.50 ± 0.88	0.29 ± 0.04	6.4
3 weeks	1.79 ± 0.56	0.12 ± 0.02	6.7
4 weeks	0.37 ± 0.08	nd[c]	—

[a] The dose was 3 mg/kg of leuprolide acetate. Each value represents the mean ± SE of five rats.
[b] Metabolite (M-I) % of the intact form.
[c] Not detectable, <0.05 ng/ml.

the 4 weeks after each injection showed a high initial peak followed by sustained levels from day 2 to the end of the fourth week (mean ± SE), 2.07 ± 0.31 (0.16–5.04) ng/ml for males and 2.18 ± 0.31 (0.50–5.18) ng/ml for females. AUCs of the serum level for the 4 weeks after the second and third injection in male and female rats were not significantly different ($P > 0.05$) from that after the first injection (Table III).

The urinary excretion is expressed as a percentage of the theoretical 1-day dose (45–55 μg/day for males, 30–45 μg/day for females) assuming that leuprolide is constantly released from the microspheres for 30 days. The percentage of the immunoreactive analogue excreted in the urine is 27–30% of the dose for 1 day in rats after s.c. injection of the analogue solution (14). As shown in Fig. 4, the percentage excreted at day 0 after each injection was 3.5–4 times higher than that calculated by the theoretical 1-day dose. The excretions at day 2 and day 7 were almost the same and about 13.5% for both male and female rats. The percentage excreted at day 14 indicated a second peak, 23.5 ± 1.2% for males and 21.6 ± 0.8% for females. The excretion decreased to about 5% at day 21 and was not detectable at day 56 (8 weeks after last dosing). These changes in excretion correlated well with the changes in the serum levels as shown in Fig. 3. As a result, the patterns of urinary excretion after each injection were almost the same. The results indicate that the absorption and excretion of the analogue are not changed and there is no accumulation of the drug in the body after repeated injection of the microspheres in both male and female rats.

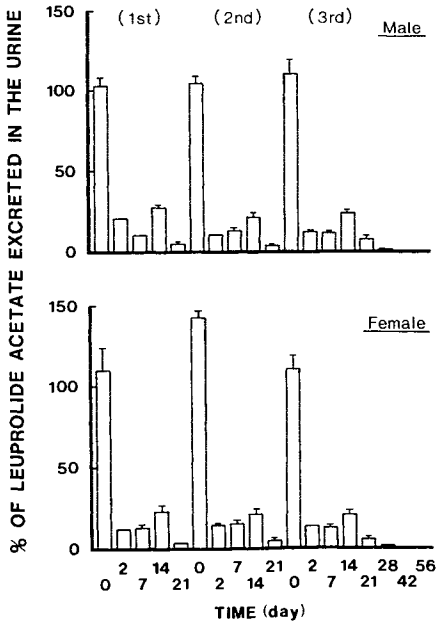

Fig. 4. Urinary excretion of leuprolide acetate after repeated s.c. injection of the microspheres (one injection every 4 weeks) in rats. The dose was 3 mg/kg of leuprolide acetate. Urinary excretion is represented as a percentage of the calculated 1-day dose of the analogue which is found in the 24-hr urine sample. Each value represents the mean ± SE of six rats.

Serum M-I After Injection of the Microspheres

The metabolites of leuprolide acetate in rats have recently been revealed to be M-I (Tyr-D-Leu-Leu-Arg-Pro-NH-C_2H_5), M-II (Tyr-D-Leu-Leu-OH), M-III (5-oxo-Pro-His-Trp-OH), and M-IV (5-oxo-Pro-His-OH) (14). Subsequently, it was clarified that our antiserum is cross-reactive with M-I (about 70% as compared to the intact form), and a more selective analytical method was developed (10). We determined the serum levels of the intact form and M-I using this new method in rats after s.c. injection of the microspheres. As shown in Table IV, the serum level of M-I was 21% of the intact drug 3 hr after injection but was very slight, 3.4 to 6.7%, 1 to 3 weeks after injection and was not detectable 4 weeks after injection. This indicates that the pharmacokinetics of leuprolide at the plateau levels after injection of this depot formulation can be assessed by conventional RIA to determine its efficacy.

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