Pharmacokinetics of Once-a-Month Injectable Microspheres of Leuprolide Acetate

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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 (leuprolarin); 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 [leuprolarin, D-Leu<sup>6</sup>-des-Gly<sup>10</sup>, NH<sub>2</sub>]-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, steroiogenesis, 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<sub>2</sub>-C<sub>3</sub>H<sub>7</sub>) 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 microspheres 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 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.
Table I. Serum Levels of Leuprolide Acetate in Rats and Dogs After i.v. and s.c. Injection of the Solution*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Rat (ng/ml)</th>
<th>Dog (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.v.</td>
<td>s.c.</td>
</tr>
<tr>
<td>5</td>
<td>142.3 (5.7)</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>123.2 (4.8)</td>
<td>46.4 (2.7)</td>
</tr>
<tr>
<td>15</td>
<td>95.4 (1.4)</td>
<td>38.3 (1.8)</td>
</tr>
<tr>
<td>20</td>
<td>77.2 (2.9)</td>
<td>44.1 (1.7)</td>
</tr>
<tr>
<td>30</td>
<td>53.6 (2.1)</td>
<td>80.2 (3.2)</td>
</tr>
<tr>
<td>45</td>
<td>46.3 (4.2)</td>
<td>80.2 (3.2)</td>
</tr>
<tr>
<td>1 hr</td>
<td>21.3 (1.9)</td>
<td>—</td>
</tr>
<tr>
<td>2 hr</td>
<td>12.3 (1.3)</td>
<td>24.0 (2.6)</td>
</tr>
<tr>
<td>3 hr</td>
<td>6.82 (1.2)</td>
<td>14.4 (1.6)</td>
</tr>
<tr>
<td>4 hr</td>
<td>1.40 (0.6)</td>
<td>6.67 (0.4)</td>
</tr>
<tr>
<td>6 hr</td>
<td>0.06 (0.1)</td>
<td>0.62 (0.3)</td>
</tr>
</tbody>
</table>

* 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-

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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*

| Parameter | Rat | Dog | Human
|-----------|-----|-----|-----|
| A (mg/ml) | 83.6 (31.3) | 293.6 (53.8) | 99.3 (13.9)
| B (mg/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)
| k₁₂ (hr⁻¹) | 2.52 (1.97) | 2.14 (1.41) | 1.29 (0.15)
| k₂₁ (hr⁻¹) | 4.55 (1.65) | 2.45 (0.84) | 0.68 (0.05)
| kₕ (hr⁻¹) | 1.59 (0.21) | 1.23 (0.18) | 0.96 (0.09)
| Vₜ (L/kg) | 0.56 (0.07) | 0.22 (0.02) | 9.18 (1.46)
| T₁/2 · α (min) | 5.5 | 7.56 | 15.5
| T₁/2 · β (hr) | 0.67 | 1.19 | 2.89

* Serum level of leuprolide acetate (Cₜ) at time t, Cₜ = A e⁻αt + B e⁻βt; transfer rate constant, k₁₂ (from central to tissue), k₂₁ (from tissue to central); elimination rate constant, kₕ; distribution volume of central, Vₜ. Each value represents the mean (SE) of five animals or six humans. Total-body clearance, Clₜot; steady-state serum level, Cₛₛ.

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

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