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# One-month release injectable microspheres of leuprolide acetate inhibit steroidogenesis and genital organ growth in rats

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## Summary

We have prepared one-month release injectable microspheres of leuprolide acetate, a highly potent analog of luteinizing hormone-releasing hormone (LH-RH), to treat endocrine-dependent tumors and endometriosis. The drug was encapsulated with a biocompatible and biodegradable polymer, copoly(DL-lactic/glycolic acid) (PLGA), by a novel in-water drying method through a (w/o)/w emulsion. The effects of this sustained-release preparation on maintaining the serum levels of the analog, on serum testosterone, and on the growth of the genital organs were evaluated in rats of different strains (Sprague–Dawley (SD) and Wistar) and ages (6 and 10 weeks). The results were compared with those obtained by constant infusion or daily pulsatile injections of the analog solution. A single injection of the PLGA microspheres sustained effective serum levels of the analog for at least 4 weeks, and persistently inhibited serum testosterone and growth of the genital organs (testis, seminal vesicle, and prostate) over 6 weeks in both strains at both ages. These antagonistic activities were almost identical to those observed after constant infusion of the analog solution, and equal or superior to those after pulsatile daily injections. A single injection of these sustained release injectable microspheres satisfactorily inhibits steroidogenesis and consequently suppresses genital organ growth; its use may eliminate the inconvenience of daily subcutaneous injections, and elevate compliance and efficacy in patients with prostatic cancer.

## Introduction

Leuprolide, (D-Leu$^6$-(des-Gly$^{10}$-$NH_2$)-LH-RH ethylamide, acetate is a superactive analog of the luteinizing hormone-releasing hormone (LH-RH) (Fujino et al., 1974). At acute doses, it stimulates the gonadotropin secretion of the pituitary, and induces ovulation. However, when administered chronically at a high dose, the analog paradoxically produces antagonistic inhibitory effects on the pituitary and gonads; these effects are attributed to down-regulation of receptors. The analog is currently being used in clinical trials to treat hormone-dependent tumors (mammary, prostate, and ovary), endometriosis, and precocious puberty (Johnson et al., 1976; Redding and Schally, 1981; Crowley et al., 1981; Lemay and Quesnel, 1982; Meldrum et al., 1982). We have previously reported a novel method preparing injectable micro-

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spheres of the analog with biocompatible and biodegradable polymers, poly(lactic acid) or copoly(DL-lactic/glycolic acid), using an in-water drying method through the process of a (w/o)/w type dual-emulsion (Okada et al., 1987; Ogawa et al., 1988). Our studies in vivo and in vitro preliminarily showed that the releasing rate and period of the analog from these microspheres was mainly controlled by the degradation of the polymers used as a cell wall matrix; this degradation directly depends on the ratio of lactic and glycolic acid in the copolymer, and on the molecular weight of polymers (Okada et al., 1985a and b; Okada et al., 1988). These studies suggested that the microspheres of the analog with PLGA(75/25)-14000 (molar ratio of lactic and glycolic acid of 75/25, average mol. wt. of around 14,000) could release the analog at the rate of pseudo-zero-order. A single injection of the microspheres sustained serum levels in rats and dogs over 4 weeks, and also suppressed steroidogenesis and growth of the genital organs.

In the present study, we further investigated the inhibitory effects of a single injection of one-month release injectable microspheres on steroidogenesis and the growth of the genital organs in rats of two different strains and ages. The results were compared with those obtained by constant infusion or daily pulsatile injections of a saline solution of the analog.

## Materials and Methods

*Animals and materials*

Sprague–Dawley (SD) and Wistar male rats purchased from Clea Japan (Tokyo, Japan) were used at 6, 8 or 10 weeks of age. Leuprolide acetate synthesized in the Central Research Division of Takeda Chemical Ind. was microencapsulated with PLGA (76.5/23.5)-12700 by an in-water drying method as previously described (Okada et al., 1987; Ogawa et al., 1988). The analog content of microspheres was 8.8%.

*RIA of serum levels of leuprolide, testosterone, and determination of genital organ weight*

Serum levels of leuprolide were determined in duplicate by the double-antibody radioimmunoassay (RIA) method as previously described (Okada et al., 1988). Testosterone was measured in duplicate by commercially available RIA kits from Green Cross Co. (Osaka, Japan, produced by Commissariat a l'Energie Atomique, France), using the single-antibody and dextran–charcoal method after ether extraction. The limit sensitivity of the assay was 5 pg of leuprolide acetate, and 6.25 pg of testosterone.

Leuprolide acetate was administered to rats (10 weeks of age) s.c. by an implanted osmotic mini-pump (Alzet Model 2002, Alza Co., Palo Alto, CA) and i.m. by the PLGA microspheres at a dose of 45 $\mu$g/day (corresponding approximately to 100 $\mu$g/kg/day at an average b.wt. of 450 g during 4 weeks of treatment). To assay serum levels of the analog, blood was collected periodically from the tail vein for 2 weeks for the mini-pump and 4 weeks for the microspheres. The serum was separated and stored at $-40\,^{\circ}$C until analyzed. To determine the dose–response relation of the suppression of serum testosterone levels, the analog was infused into 4 SD rats (8 weeks of age) with the implanted mini-pump at doses of 0.1, 1, 10 and 100 $\mu$g/day (corresponding approximately to 0.3, 3, 30 and 300 $\mu$g/kg/day), and blood was collected from the tail vein 2, 4 and 6 h, and 1, 2, 3, 4, 5 and 6 days after the implantation. In the study on the inhibition of growth of the genital organs, the right testis, the whole prostate glands, except the coagulate glands, and both seminal vesicles of 5 treated rats were carefully removed 2 weeks after the drug was infused with the mini-pump, or injected s.c. by way of the microspheres. Each genital organ was weighed and compared with those of untreated rats of the same age. The doses were 0.045, 0.45, 4.5 and 45 $\mu$g/day for the mini-pump (corresponding approximately to 0.1, 1, 10 and 100 $\mu$g/kg/day at an average b.wt. of 450 g during 4-weeks of treatment), and 0.45, 4.5 and 45 $\mu$g/day as leuprolide acetate for the microspheres.

After daily s.c. injections of the analog solution and a single injection of the microspheres, the serum testosterone levels during 2-weeks of treatment and the weight changes of the genital organs 1 and 2 weeks after treatment were determined in 10-week-old rats. Blood was periodically collected

from the abdominal artery under ether anesthesia, and the serum was stored at −40 °C until assayed. The doses of leuprolide acetate were, for the saline solution of the analog (100 μg/ml), 0.45 ml/day for SD rats and 0.35 ml/day for Wistar rats, and for the microspheres 1.35 mg for SD rats and 1.05 mg for Wistar rats; these doses corresponded to an approximate average dose of 100 μg/day/day, estimated by an average b.wt. of 450 g and 350 g, respectively, during 4 weeks of treatment.

To study the differences between pharmacologic activities in the two different strains, the microspheres were administered i.m. to 10-week-old animals at a dose corresponding to 100 μg/kg/day of leuprolide acetate, and the serum testosterone levels were measured for 8 weeks following injection and the genital organs were weighed for 6 weeks. The adrenal glands were removed and weighed in Wistar rats during this 6 week period.

The microspheres were injected i.m. into 6-week- and 10-week-old SD rats at a dose corresponding to 100 μg/kg/day of the analog (0.9 mg and 1.35 mg/rat, respectively), and the pharmacologic effects were determined as described above to evaluate the influence of age.

## Results

The serum levels of leuprolide acetate in rats after continuous infusion by implantation of the osmotic mini-pump attained a steady-state level, about 4 ng/ml, in 3 h, and were maintained for 2 weeks (Fig. 1). However, at 6 h and 1 day after the administration the levels unexpectedly dropped. After an initial higher level, 20.6 ± 5.6 ng/ml (mean ± S.E.M.) at 3 h, the serum levels after microspheres were injected i.m. were also maintained at a plateau level of near 4 ng/ml for 2 weeks. The levels gradually declined to 1 ng/ml at 4 weeks after, and the drug was still detectable after 6 weeks (0.34 ng/ml).

When the analog was infused continuously, the testosterone serum levels were elevated immediately after the infusion was started, but decreased gradually to significantly below the normal levels of untreated control rats 3 days after the infusion was started at doses of over 10 μg/day (corresponding to 30 μg/kg/day) (Table 1, Fig. 2). At a dose of 3 μg/kg/day this inhibitory effect slowly occurred, but was discernible 5 days after the infusion was started; the effect was scarcely observed at 0.3 μg/kg/day.

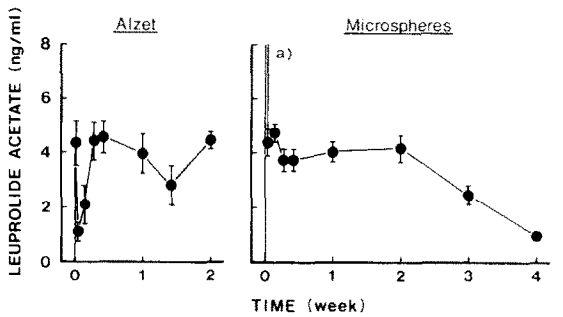


Fig. 1. Serum levels of leuprolide acetate in rats (10 weeks of age) after continuous infusion by an osmotic minipump (Alzet) or a single injection of PLGA microspheres at a dose corresponding to 100 μg/kg/day. Each point represents the mean ± S.E.M. of 5 rats. (a) $C_{3h} = 20.6 \pm 5.6$ ng/ml.

Fig. 3 shows the inhibitory responses of the genital organs at different doses of the analog 2 weeks after infusion by the mini-pump or a single injection of microspheres. The growth of the testis (left open bars) was significantly inhibited by both

TABLE 1

*Serum testosterone levels in rats (8 weeks of age) during 6-days continuous infusion of leuprolide acetate at 4 different doses* (ng/ml)

Each value represents the mean (S.E.M.) of 4 rats.

| Time | Dose of leuprolide acetate (μg/kg/day) | | | |
|---|---|---|---|---|
| | 0.3 | 3 | 30 | 300 |
| 0 h [a] | 2.03 (0.20) | 1.51 (0.36) | 2.47 (0.42) | 1.48 (0.21) |
| 2 | 3.53 (2.38) | 13.3 (5.17) | 26.6 (5.06) | 24.6 (2.33) |
| 4 | 1.87 (0.88) | 27.4 (6.05) | 14.6 (1.75) | 17.4 (2.52) |
| 6 | 5.55 (4.33) | 17.4 (4.16) | 11.7 (2.22) | 10.5 (1.92) |
| 1 day | 5.66 (2.20) | 9.40 (1.88) | 7.22 (1.48) | 6.07 (0.57) |
| 2 | 3.16 (1.51) | 4.65 (1.21) | 2.93 (0.45) | 2.50 (0.66) |
| 3 | 2.46 (0.72) | 1.83 (0.27) | 1.08 (0.11) | 0.96 (0.21) |
| 4 | 2.15 (0.60) | 1.48 (0.17) | 0.89 (0.13) | 0.77 (0.18) |
| 5 | 3.70 (1.61) | 1.06 (0.07) | 0.73 (0.06) | 0.65 (0.12) |
| 6 | 1.32 (0.54) | 1.20 (0.13) | 0.56 (0.08) | 0.56 (0.09) |

[a] The mean (SE) of initial levels of serum testosterone: 1.87 (0.17) ng/ml, $n = 16$.

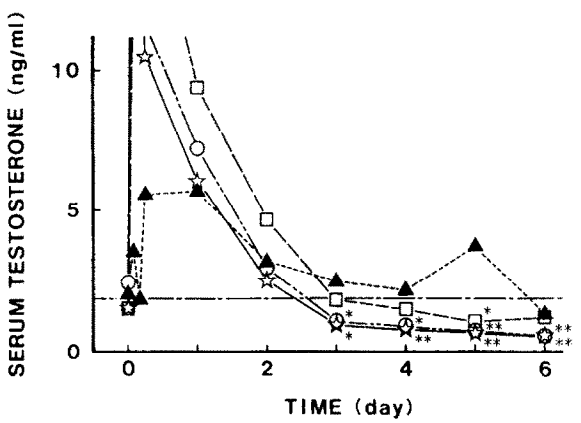


Fig. 2. Serum testosterone levels in rats (8 weeks of age) during 6 days of continuous infusion of leuprolide acetate by the mini-pump at 4 different doses: 0.3 (▲), 3 (□), 30 (○), and 300 (☆) μg/kg/day. Each point represents the mean of 4 rats. Significantly suppressed from the untreated control by Student's *t*-test (* $P < 0.05$; ** $P < 0.01$). The serum levels 2 and 4 h after implantation are shown in Table 1.

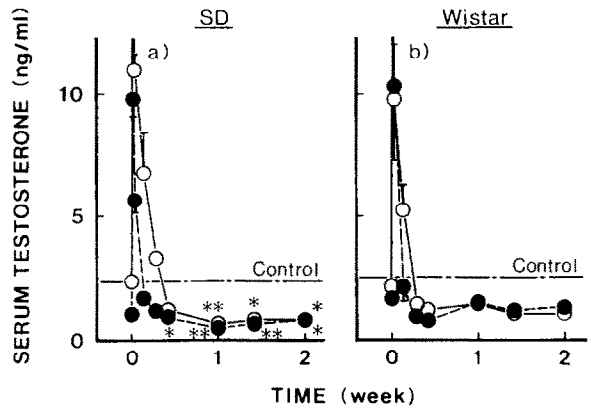


Fig. 4. Serum testosterone levels in rats (10 weeks of age) after daily injections of leuprolide saline solution (●) or a single injection of PLGA microspheres (○) at a dose corresponding to 100 μg/kg/day as leuprolide acetate. Serum levels 2 h after the injection of the daily solution were plotted between 1 day and 2 weeks. Each point represents the mean ± S.E.M. of 5 rats. Significantly different from untreated control (2.47 ± 0.32 ng/ml for Wistar rats, $n = 35$; 2.36 ± 0.18 ng/ml for SD rats, $n = 55$) by Student's *t*-test (* $P < 0.05$; ** $P < 0.01$). (a) $C_{3h}$ = 15.3 ± 2.4 ng/ml for the microspheres. (b) $C_{3h}$ = 12.9 ± 2.0 ng/ml for the microspheres, 12.6 ± 3.7 ng/ml for the daily solution.

treatments at doses over 1 μg/kg/day; the inhibition increased dose-dependently. The growth of the seminal vesicle (right hatched bars) was reduced strikingly by both treatments at doses of 10 and 100 μg/kg/day (significantly different from untreated control, $P < 0.01$ or $P < 0.05$). The prostate (central dotted bars) did not readily decrease after either treatment, but was obviously depressed at a dose of 100 μg/kg/day. A single injection of the leuprolide microspheres at a dose

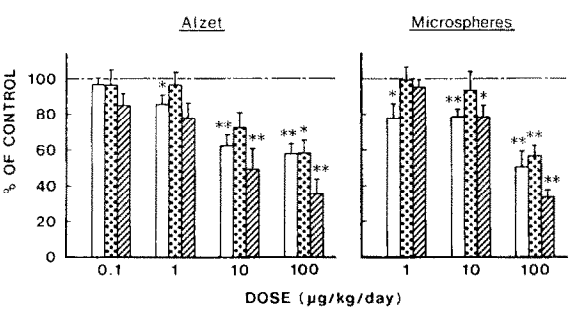


Fig. 3. Weight changes of testis (open bar), prostate (dotted bar), and seminal vesicle (hatched bar) in rats (10 weeks of age) 14 days after continuous infusion of leuprolide acetate by the mini-pump or a single injection of PLGA microspheres. Each bar represents the mean ± S.E.M. of 5 rats. Significantly different from untreated control by Student's *t*-test (* $P < 0.05$; ** $P < 0.01$).

of 100 μg/kg/day severely inhibited the growth of the genital organs (significantly different from untreated control, $P < 0.01$); the extent of suppression was similar or superior to that observed after constant infusion by the mini-pump.

The serum testosterone levels in SD and Wistar rats after a single administration of the microspheres and daily injections of the saline solution of the analog at a dose of 100 μg/kg/day were elevated at the start of treatment (Fig. 4), but was obviously reduced during 3–14 days of treatment to a quarter or half of the normal levels in untreated rats 10–18 weeks of age (2.36 ± 0.18 ng/ml for SD, $n = 55$; 2.47 ± 0.32 ng/ml for Wistar, $n = 35$), to the same extent by both administrations. As shown in Fig. 5, all 3 genital organs in both strains were significantly suppressed 1 and 2 weeks after administration of the microspheres and daily injections of the solution ($P < 0.01$). In Wistar rats 2 weeks after treatment, a more persistent inhibition of the growth of the prostate gland (76.1% against the daily solution treatment, $P < 0.02$) and seminal vesicle (61.8%, $P < 0.02$)

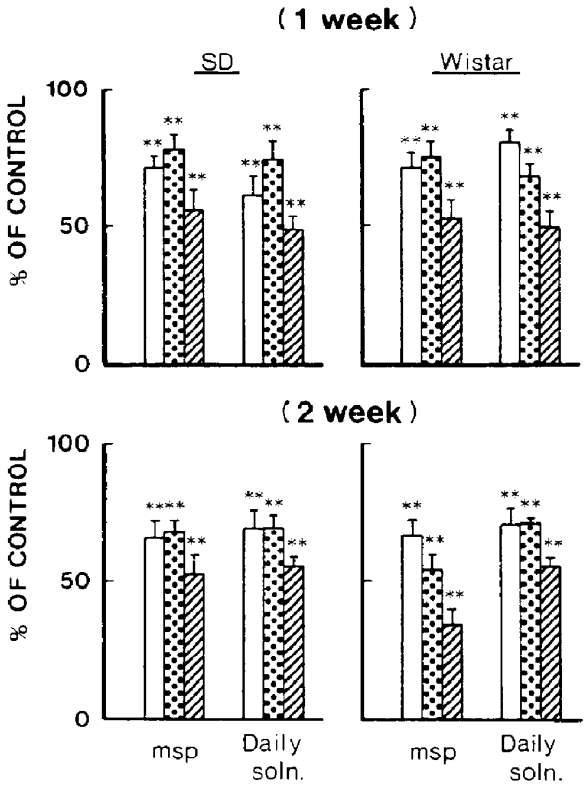


Fig. 5. Weight changes of testis (open bar), prostate (dotted bar), and seminal vesicle (hatched bar) 1 and 2 weeks after daily injections of a leuprolide saline solution or a single injection of PLGA microspheres at a dose corresponding to 100 $\mu$g/kg/day. Each bar represents the mean $\pm$ S.E.M. of 5 rats. Significantly different from untreated control by Student's $t$-test (** $P < 0.01$).

was achieved by the microsphere-treatment than by daily injection of the analog solution (Fig. 5).

Further study on the pharmacological activities of a single injection of the microspheres indicated a similar drastic inhibition of the serum testosterone in both strains (10 weeks of age) 3 days to 6 weeks after the injection at a dose corresponding to 100 $\mu$g/kg/day (* $P < 0.05$ or ** $P < 0.01$) (Fig. 6). Fig. 7 shows the inhibitory effects on the growth of the 3 genital organs in SD and Wistar rats, and the response of the adrenal gland in the Wistar strain. The testis in both strains decreased rapidly following treatment, and the weight was highly significantly suppressed between 3 days and 6 weeks of treatment of the untreated control ($P < 0.01$); this inhibition was

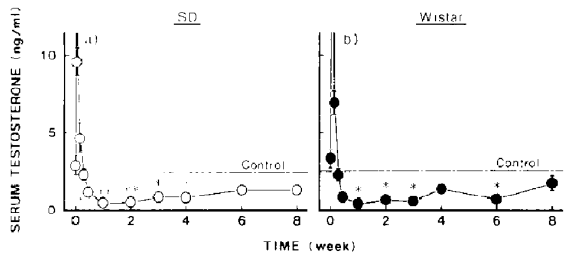


Fig. 6. Serum testosterone levels in SD (○) and Wistar (●) rats (10 weeks of age) after a single injection of leuprolide PLGA microspheres at a dose corresponding to 100 $\mu$g/kg/day. Each point represents the mean $\pm$ S.E.M. of 5 rats. Significantly different from untreated control by Student's $t$-test (* $P < 0.05$; ** $P < 0.01$). (a) $C_{3h} = 16.1 \pm 1.6$ ng/ml for SD rats. (b) $C_{3h} = 24.0 \pm 3.2$ ng/ml, $C_{6h} = 18.8 \pm 3.3$ ng/ml for Wistar rats.

maintained for 6 weeks at about 50% of the control level in both strains. The weights of the seminal vesicle and prostate were not affected, or increased a little 3 days after injection, but markedly decreased by 20–70% of untreated control between 1 week and 6 weeks ($P < 0.05$ or $P < 0.01$). The maximum inhibition in both genital organs was larger in the Wistar strain; the sensitivity of the inhibitory response to the analog might be higher in this strain. Incidentally, weight change

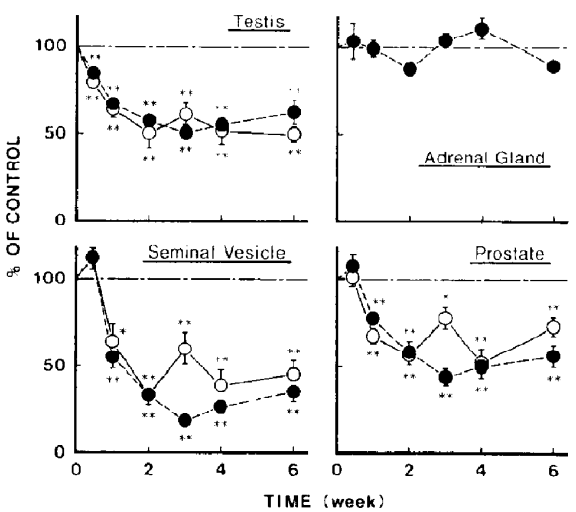


Fig. 7. Weight changes of the genital organs and adrenal gland in SD (○) and Wistar (●) rats (10 weeks of age) after a single injection of leuprolide PLGA microspheres at a dose corresponding to 100 $\mu$g/kg/day. Each point represents the mean $\pm$ S.E.M. of 5 rats. Significantly different from untreated control by Student's $t$-test (* $P < 0.05$; ** $P < 0.01$).

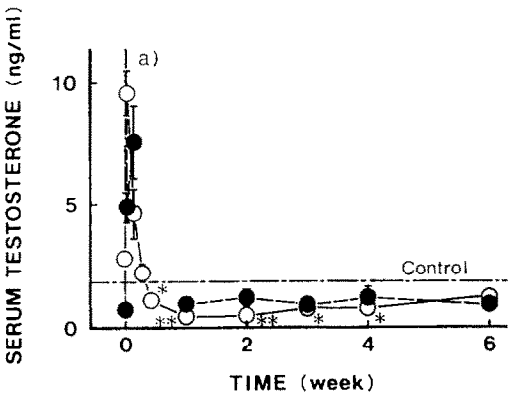


Fig. 8. Serum testosterone levels in 6- (●) and 10- (○) week-old rats after a single injection of leuprolide PLGA microspheres at a dose corresponding to 100 μg/kg/day. Each point represents the mean ± S.E.M. of 5 rats. Significantly different from untreated control by Student's *t*-test (* $P < 0.05$; ** $P < 0.01$). (a) $C_{3h} = 13.1 \pm 3.6$ ng/ml for 6-week-old rats, $16.1 \pm 1.6$ ng/ml for 10-week-old rats.

of the adrenal gland in the Wistar rats was not affected by the microspheres.

Figs. 8 and 9 indicate the influences of age on the inhibition of the serum testosterone and the growth of genital organs following treatment with the microspheres. The testosterone serum level of untreated control rats between 6 and 14 weeks of age was $1.86 \pm 0.15$ (mean ± S.E.M. $n = 50$), and significantly lower than that of the older animals described above between 10 and 18 weeks of age ($P < 0.05$). Serum testosterone in 6-week-old rats at doses corresponding to 100 μg/kg/day was also reduced to half of the control level for 1–6 weeks after the initial flare-up; however, this suppression did not produce a significantly different value from that of the control ($P > 0.05$) (Fig. 8). Although the rate of suppression was not obvious because no determinations were made on days 2 and 3 after the injection, the extent of inhibition tended to be a little weaker than that in the 10-week-old rats. As shown in Fig. 9, the growth of the testis was identically suppressed at both ages, and was significantly different from the control rats by 3 days after the start of treatment ($P < 0.05$ or $P < 0.01$). Larger initial flare-up responses on the seminal vesicle and prostate glands in the younger animals (6 weeks of age) were obtained by a single injection of the microspheres, and were followed by a retarded inhibition about 1 or 2 weeks behind that in the old animals. Nevertheless, a similar remarkable suppression was finally achieved in animals of both ages from 2 or 3 to 6 weeks of treatment.

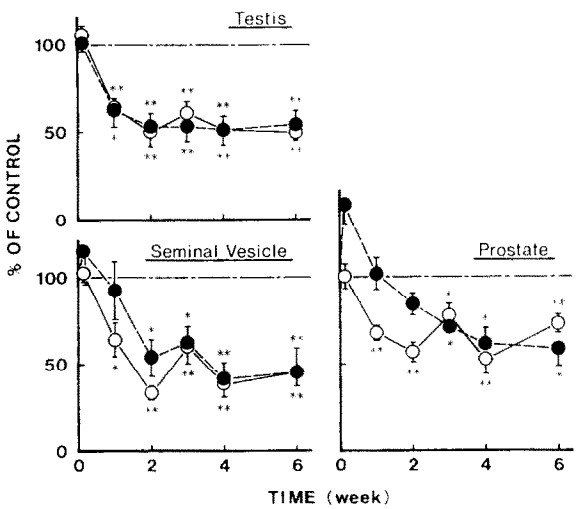


Fig. 9. Weight changes of genital organs in 6- (●) and 10- (○) week-old rats after a single injection of leuprolide PLGA microspheres at a dose corresponding to 100 μg/kg/day. Each point represents the mean ± S.E.M. of 5 rats. Significantly different from untreated control by Student's *t*-test (* $P < 0.05$; ** $P < 0.01$).

## Discussion

The sustained serum levels and pharmacologic effects after a single injection of one-month release microspheres of leuprolide acetate were evaluated and compared with those obtained by the constant infusion by a osmotic mini-pump, Alzet. With constant infusion, plateau serum levels of the analog were maintained at about 4 ng/ml during 2 weeks of treatment, although two unexplained drops occurred at 6 h and 1 day. A single injection of the microspheres also maintained the same plateau serum levels of the analog at about 4 ng/ml from 6 h to 2 weeks after the injection. This serum level was well-sustained for another 2 weeks (Fig. 1). The initial burst from the microspheres for a very short period immediately following the start of treatment could not be avoided.

However, this initial burst must be considered to be fairly small, because it is generally known that the release of hydrophilic compounds from monolithic microspheres shows a much larger burst (Chang, 1976; Tice and Cowsar, 1984). The small size of the burst might result from our novel in-water drying method through a (w/o)/w emulsion (Okada et al., 1987), which produces microspheres in which drug is rigidly entrapped likely attributed to the addition of a drug retaining compound and ion interaction between the positive charged analog and negatively charged cell wall polymer of the microspheres. The progressive decline of the serum levels from 2 weeks after the injection seems to result from a slight decrease in the release rate of the drug from the microspheres and an increase of b.wt.; it increased 1.3–1.4 times at 4 weeks after the start of treatment. The total body clearance ($Cl_T$) is 935.5 ml/h/kg, calculated using the dose (100 $\mu$g/kg) and infinite area under the serum level–time curve for 0 to $\infty$ (106.9 ng · h/ml) in rats after s.c. injection of the analog solution (unpublished data). The release rate ($k$) of drug from this microspheres is 2.8% of dose/day, about 3.5 $\mu$g/h/kg (Okada et al., 1988). Thus, the theoretical steady plateau level ($C_{ss}$) of the analog is calculated as:

$$C_{ss} = k/Cl_T = 3.5 \times 10^3/935.5 = 3.7 \text{ ng/ml}$$

The serum levels after the injection of the microspheres agree well with this calculated value. A single injection of the microspheres provided prolonged effective serum levels, more than 1 ng/ml, of the analog for cancer treatment for over 4 weeks (Okada et al., 1983b). Parenteral injection systems of similar LH-RH analogs have been reported to be prepared by a phase separation method using 3 kinds of organic solvent or oil, and to release the analog for about 3 weeks (Redding et al., 1984; Sanders et al., 1984). Our preparation method has advantages in that only one volatile organic solvent is used in the preparation and is easily removed after encapsulation. The results of the release study indicate that the microspheres provide more prolonged and efficient serum levels for 4 weeks of treatment.

The dose-defining study using an infusion mini-pump and microspheres indicated the testosterone was elevated transiently, a "flare-up", at the start of treatment, and was then suppressed markedly 3 days after infusion at a dose of over 10 $\mu$g/rat/day (corresponding to about 30 $\mu$g/kg/day) (Fig. 2). The growth of the genital organs – the right testis, both seminal vesicles, and both ventral and dorsal prostate glands except the coagulate glands – was significantly suppressed in a similar manner following administration by infusion mini-pump or microspheres; the testis, seminal vesicle and prostate were suppressed at doses over 1 $\mu$g/kg/day, 10 $\mu$g/kg/day, and 100 $\mu$g/kg/day, respectively. All 3 genital organs were significantly suppressed at a dose of 100 $\mu$g/kg/day ($p < 0.01$); this effective dose coincides with that in treating the mammary tumor (Okada et al., 1983b) and endometriosis (Okada et al., 1988) in our previous studies.

The pharmacologic effects obtained by daily pulsatile injections of the analog solution and a single injection of the microspheres at a dose of 100 $\mu$g/kg/day were almost the same in the two different strains of rats for 2 weeks (Figs. 4, 5). However, much stronger suppressive effects were exerted by the microspheres in Wistar rats 2 weeks after administration (significantly different in the prostate and seminal vesicle, $P < 0.02$). Thus, the chemical castration caused by an injection of the microspheres might be equal or superior to that obtained by pulsatile daily injections of the analog solution. Microspheres of the analog cause more remarkable regression of experimental endometriosis in female rats than does a daily s.c. injection of the solution (Okada et al., 1988). The results of Fraser and Sandow (1985), and those of our previous studies (Okada et al., 1983a) have also indicated that a constant infusion or repeated vaginal administration of the LH-RH analog provides sustained serum levels and achieves stronger antagonistic inhibition on the gonadal–pituitary systems than pulsatile s.c. injection. The consistent down-regulation effect is better achieved by continuous hits of the target cell by potent LH-RH agonists.

The testosterone serum levels after a single injection of microspheres were dramatically reduced to the same extent in the both strains of

rats for 4 or 6 weeks after injection (Fig. 6). The growth of 3 genital organs was also strikingly suppressed by a single injection in both strains; the treatment with the microspheres likely provided a stronger inhibition of the seminal vesicle and prostate in the Wistar rats (Fig. 7). On the other hand, chronic leuprolide treatment had no effects on the morphologic changes of the adrenal gland, which is another endocrine organ partially secreting sex steroids and possibly controlled not by gonadotropins.

The inhibitory responses in rats were certainly affected by the age of the animals at the start of treatment. The serum testosterone levels of 6-week-old rats at the commencement of treatment were significantly lower than the average of those in 6–18-week-old animals ($P < 0.05$). The suppressed serum testosterone during treatment by microspheres was half of the control level, but the difference was not significant ($P > 0.05$), tended to be slightly weaker than that in 10-week-old rats. However, the growth of testis was severely inhibited to the same degree in the different aged animals 1–6 weeks after injection. In the seminal vesicle and prostate, in which a large initial flare-up was obviously produced, there was no significant inhibition for 1 week seminal vesicle, and for at least 2 weeks prostate after treatment (Fig. 9). Finally, the microsphere injection caused a persistent inhibition even in young animals. The use of older animals, such as 10-week-old rats, may be prefered to precisely assess responses; older animals are more appropriate and also more closely reflect clinical usage because prostate tumors occur more frequently in the elderly patient.

The use of superactive LH-RH agonists to treat hormone-dependent cancers and gynecologic diseases opens up exciting new therapeutic possibilities; few severe side-effects have been reported in recent numerous clinical studies (Smith, 1987; Lemay, 1987). The initial flare-up in the first few days of treatment with LH-RH potent analogs may occur because of transient androgen stimulation, and might possibly result in the exacerbation of the disease-related symptoms at first. It has been suggested that combined therapy with anti-androgens, such as cyproterone acetate and flutamide, may reduce the initial problems and increase the antitumor response of LH-RH agonists (Labrie et al., 1986). In our preliminary study, the combined treatment of the microspheres with an anti-androgen, oxendolone, tended to reduce the initial flare-up (unpublished data). Oxendolone, 16$\beta$-ethyl-17$\beta$-hydroxy-4-estren-3-one, is clinically used to treat prostatic hyperplasias and acts by competing with the androgen receptor (Nakayama et al., 1979).

In summary, a single injection of leuprolide microspheres with copoly(DL-lactic/glycolic acid) (76.5/23.5, molar ratio of lactic and glycolic acid; mol.wt., 12,700) provided sustained effective serum levels of the analog for at least 4 weeks. The persistent inhibition of serum testosterone, and of the growth of the genital organs (testis, seminal vesicle, and prostate) were produced by the single injection for over 6 weeks in rats of two different strain (SD and Wistar) and ages (6 and 10 weeks). These antagonistic pharmacologic activities by the injection resulting from the suppression of steroidogenesis were almost identical to those obtained by constant infusion of an analog solution, and the same as or superior to those obtained after pulsatile daily injections of the analog solution; such a response might produce regression of a hormone-dependent tumor like a prostate tumor.

A single administration of one-month release injectable microspheres of the analog satisfactorily inhibited steroidogenesis and consequently suppressed the growth of the genital organs; such a treatment may eliminate the inconvenience of daily subcutaneous injections, and may elevate compliance and efficacy in patients with prostatic cancer.

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