

Long-acting delivery systems for peptides: Inhibition of rat prostate tumors by controlled release of [D-Trp⁶]luteinizing hormone-releasing hormone from injectable microcapsules

(luteinizing hormone-releasing hormone analogs/prostate cancer/tumor weight and volume reduction/monthly injection of peptides)

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ABSTRACT Intramuscular injection of [6-D-tryptophan]-luteinizing hormone-releasing hormone ([D-Trp⁶]LH-RH) in microcapsules of poly(DL-lactide-co-glycolide), designed to release a controlled dose of the peptide over a 30-day period, decreased the weights of androgen-dependent Dunning prostate tumors in rats and suppressed serum testosterone levels more effectively than daily subcutaneous administration of equivalent or double doses of unencapsulated [D-Trp⁶]LH-RH. The microcapsules or daily injections of [D-Trp⁶]LH-RH also significantly decreased tumor volumes. Microcapsules of [D-Trp⁶]LH-RH or related analogs that can be injected once a month should make the treatment of patients with prostate carcinoma and other neoplasms or disorders more convenient and efficacious.

A marked inhibition of pituitary and gonadal function that occurs after chronic administration of the D-Trp⁶ analog of luteinizing hormone-releasing hormone (LH-RH) and other LH-RH agonists (1-9) leads to a chemical castration and makes possible an additional approach for the treatment of sex hormone-dependent tumors (10, 11). In previous studies we have shown that chronic administration of [D-Trp⁶]LH-RH suppressed prostate tumor growth in two different rat models (12, 13). In rats with prostate tumors treated with [D-Trp⁶]LH-RH, tumor weights and volumes, the weights of accessory sex organs, and serum levels of testosterone and prolactin were greatly reduced as compared to controls (12, 13). These studies demonstrated the potential clinical efficacy of [D-Trp⁶]LH-RH in the treatment of prostate carcinoma and other hormone-dependent tumors in man. Subsequent clinical trials documented marked improvement in patients with stage C or D prostate carcinoma after prolonged treatment with [D-Trp⁶]LH-RH, [D-Ser(Bu⁶)]LH-RH ethyl amide, [D-Leu⁶]LH-RH ethyl amide, and other agonistic analogs of LH-RH (14-21, §). In these trials, superagonists of LH-RH were given daily by the subcutaneous (s.c.) or intranasal route.

The use of [D-Trp⁶]LH-RH and related analogs for the treatment of endocrine-dependent tumors and other disorders would be greatly enhanced by practical delivery systems capable of maintaining controlled levels of the peptide over an extended period of time. The present modes of administration [intravenous, s.c., intramuscular (i.m.), or intranasal] lead to an initial delivery of the analog to the target tissue in high concentrations, but thereafter its levels in blood continuously decline. Depending on the route of administration and the vehicle, the duration of delivery may last from a few minutes to several hours. These modes of administration, with fluctuating blood levels of the peptide,

may not achieve the optimal pharmacological effects. Consequently, higher-dosage regimens and greater frequency of administration may be needed to obtain these effects. A peptide administered by a controlled delivery system that would lead to a constant release over a prolonged period of time could provide a continuous biological effect. Recently, a polymer of poly(DL-lactide-co-glycolide) (PLG) has been used to formulate microcapsules with steroids for long-acting contraceptive delivery systems (22-24). This polymer is biodegradable and compatible with living tissue. We decided to use a similar PLG formulation for microcapsules containing [D-Trp⁶]LH-RH. The microcapsules were designed for controlled release of the LH-RH analog over a 30-day period. In the present study we compared the effects of chronic s.c. injections of unencapsulated [D-Trp⁶]LH-RH with those produced by a preprogrammed release of [D-Trp⁶]LH-RH from a microcapsule formulation on the growth of the androgen-dependent Dunning prostate tumors in rats.

MATERIALS AND METHODS

Male (Copenhagen × Fisher) F₁ rats bearing the androgen-dependent well-differentiated R3327H Dunning rat prostate adenocarcinoma were provided by Norman Altman (Papanicolaou Cancer Research Institute, Miami, FL). Approximately 150 days after the transplantation, the tumors were palpable and the experiment began. Tumors were measured weekly with microcalipers and tumor volumes were calculated as previously described (12, 13). One group of animals was injected s.c. twice a day (b.i.d.) with 12.5 or 25 μg of [D-Trp⁶]LH-RH in 200 μl of saline. Another group of tumor-bearing rats received i.m. a microcapsule formulation of [D-Trp⁶]LH-RH. The microcapsules, in aliquots of 33 mg calculated to release a dose of about 25 μg/day for 30 days, were suspended in disposable syringes in 0.7 ml of injection vehicle containing 2% carboxymethylcellulose and 1% Tween 20 in water. The suspension was mixed thoroughly on a Vortex mixer and injected through an 18-gauge needle deep into the thigh muscle of rats. Control tumor rats were injected with the vehicle. At various time intervals, blood was removed from the jugular vein of rats for hormone analyses. After 30 days of treatment the rats were sacrificed by decapitation 1-2 hr after the last s.c. injection of the peptide. Trunk blood was collected and serum was separated for further analyses. Tumors and various organs were removed, cleaned, carefully weighed, and then quickly frozen on dry ice. All data are expressed as the mean ± SEM. Statistical analyses were performed using a computer-assisted program for nonpara-

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Abbreviations: LH-RH, luteinizing hormone-releasing hormone; b.i.d., twice a day.

§Glode, L. M. (1982) Proc. 18th Ann. Mtg. Amer. Soc. Clin. Oncology, p. 110.

metric rank-sign test of Wilcoxon (25) or Duncan's new multiple-range test (26).

Serum levels of prolactin, growth hormone, and testosterone were measured by radioimmunoassays (RIAs) as previously described (12, 13). The levels of [D-Trp⁶]LH-RH were measured by an RIA developed in this laboratory, using an antiserum generated against [D-Trp⁶]LH-RH (27).

[D-Trp⁶]LH-RH was synthesized by solid-phase methods (28) and supplied by Debiopharm (Lausanne, Switzerland). Microcapsules of [D-Trp⁶]LH-RH were prepared by a phase-separation process. The resulting product was a free-flowing powder of spherical particles consisting of [D-Trp⁶]LH-RH (2% wt/wt), distributed within a polymeric matrix of 53:47 (mol %) poly(DL-lactide-co-glycolide) (98% wt/wt) with an inherent viscosity of 0.7 dl/g. Prior to their administration the microcapsules were loaded in disposable syringes and sterilized with a 2-Mrad (0.02-mega gray) dose of γ radiation. This procedure did not affect the biological activity of the peptide.

RESULTS

The photomicrograph (Fig. 1), obtained by scanning electron microscopy, shows that the microcapsules were 50 μ m or less in diameter. The surfaces of the microspheres were smooth, indicating that a continuous coating of polymer was present. A typical cross-sectional view of [D-Trp⁶]LH-RH microcapsules is shown in Fig. 2.

In the first experiment one group of tumor rats was injected i.m. with microcapsules designed to release [D-Trp⁶]LH-RH at a controlled rate of about 25 μ g over a 24-hr period for 30 days, while another group was given unencapsulated [D-Trp⁶]LH-RH s.c. at a dose of 12.5 μ g b.i.d. Control tumor rats were given s.c. injections of the vehicle. In rats injected with microcapsules tumor weights were decreased by 81% ($P = 0.01$), whereas in the group injected daily s.c. there was only a 38% reduction in tumor weights as compared to controls (Table 1). Nonparametric analysis of the individual responses of tumors (differences from the initial volume expressed as percentage change) indicated that [D-Trp⁶]LH-RH, given either as a s.c. injection or by i.m. injection of microcapsules, significantly decreased tumor volumes (Table 1). Chronic treatment with [D-Trp⁶]LH-RH by either route also significantly decreased ventral prostate weights as

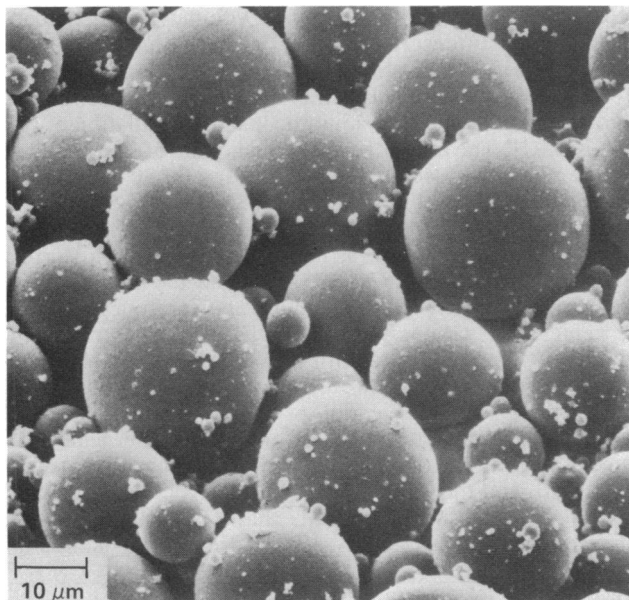


FIG. 1. Scanning electron microscopy of microcapsules of [D-Trp⁶]LH-RH in poly(DL-lactide-co-glycolide).

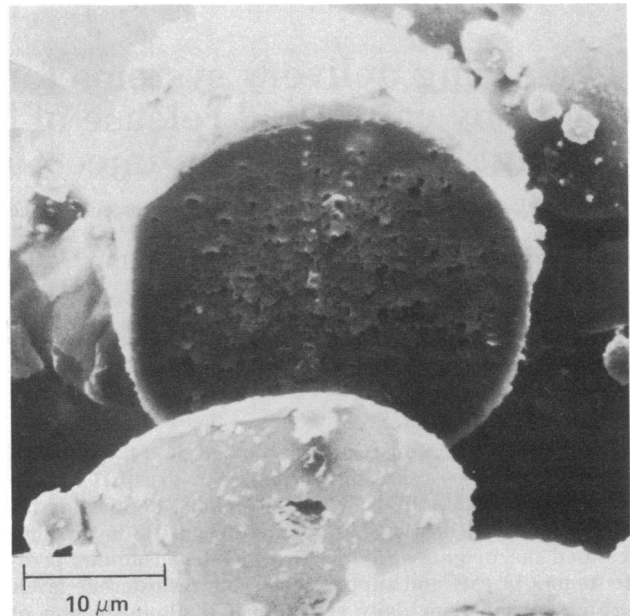


FIG. 2. Typical cross-sectional view of [D-Trp⁶]LH-RH microcapsules by scanning electron microscopy.

compared to controls. Testes weights in the group given microcapsules and injected s.c. were reduced by 60% ($P = 0.01$) and 42% ($P = 0.01$), respectively, but neither group showed significant changes in body weight or the weights of the anterior pituitary gland or adrenal gland (Table 1).

In a second experiment, using the same design as in the first, [D-Trp⁶]LH-RH was given s.c. at a dose of 25 μ g b.i.d., double that of the microcapsule formulation. Tumor weights in the microcapsule-injected group were decreased by 82% ($P = 0.01$); a reduction similar to that seen in the first experiment (Table 1). Although tumor weights in the group injected s.c. were reduced by 54% as compared to controls ($P = 0.05$), doubling the s.c. dose of [D-Trp⁶]LH-RH was no more effective in decreasing tumor weight than the microcapsules. Again, on a percentage change basis both treatments reduced tumor volume. Ventral prostate weights were decreased in the group treated with microcapsules by 87% ($P = 0.01$) and in the s.c. injected group by 84% ($P = 0.01$), while the weights of the testes were reduced by 59% ($P = 0.01$) in the former group as compared to 52% ($P = 0.01$) in the latter. Body weights, anterior pituitary, and adrenal weights were not altered by this treatment as compared to control rats.

Serum testosterone levels in rats with prostate tumors injected with [D-Trp⁶]LH-RH are shown in Table 2. In experiment 1, 5 days after the injection of microcapsules, testosterone levels were suppressed by more than 90% as compared to control tumor rats. By day 19, testosterone fell to undetectable levels, and it was still undetectable by our RIA methods on day 30. In rats given [D-Trp⁶]LH-RH s.c., testosterone fell by more than 70% after 5 days of treatment but remained at detectable levels throughout the experiment. In the second experiment, testosterone levels were suppressed but still detectable after 30 days of treatment in rats injected by the s.c. route. In the group treated with microcapsules, testosterone was completely suppressed at 30 days, as in the first experiment. In both experiments [D-Trp⁶]LH-RH, administered by either route, significantly decreased serum prolactin levels but failed to alter growth hormone.

Weekly measurements of plasma [D-Trp⁶]LH-RH by RIA in rats injected with microcapsules indicated levels up to 1000 pg/ml during the 30 days of treatment. The profiles of [D-Trp⁶]LH-RH release from microcapsules and resulting

Table 1. Effect of monthly microcapsules or daily s.c. injections of [D-Trp⁶]LH-RH on the growth of the Dunning R3327H prostate tumor in rats

Treatment	Dose	Ventral prostate, mg*	Testes, g*	Tumor weight, g*	Change in tumor volume, %†
Experiment 1					
Controls	—	419 ± 39	3.14 ± 0.07	0.357 ± 0.118	157 ± 23
Microcapsules once a month, i.m.	25 µg/day	47 ± 5‡	1.26 ± 0.06‡	0.068 ± 0.032‡	81 ± 17‡
[D-Trp ⁶]LH-RH b.i.d., s.c.	12.5 µg	88 ± 7‡	1.83 ± 0.07‡	0.220 ± 0.063	68 ± 9‡
Experiment 2					
Controls	—	337 ± 48	2.81 ± 0.13	10.06 ± 1.6	228 ± 42
Microcapsules once a month, i.m.	25 µg/day	44 ± 5‡	1.15 ± 0.08‡	1.86 ± 0.95‡	106 ± 18§
[D-Trp ⁶]LH-RH b.i.d., s.c.	25 µg	54 ± 5‡	1.36 ± 0.14‡	4.66 ± 1.3§	85 ± 30‡

Results are mean ± SEM; there were 7–10 animals per group.
 *Significance determined by Duncan's new multiple-range test.
 †Significance determined by rank sign test of Wilcoxon.
 ‡P < 0.01 compared to controls.
 §P < 0.05 compared to controls.

blood levels in normal and experimental rats bearing prostate tumors is reported elsewhere (27).

DISCUSSION

The androgen dependency of the Dunning prostate adenocarcinoma R3327H is well documented (29–31). The most effective means of inhibiting tumor growth in the Dunning rat model is hypophysectomy, alone or in combination with orchietomy (32). The suppressive effect of [D-Trp⁶]LH-RH on testosterone, gonadotropin, and prolactin levels (12, 13) can therefore mimic, in part, the effect of hypophysectomy and castration.

Our findings indicate that a single i.m. administration of microcapsules of [D-Trp⁶]LH-RH designed for controlled release can suppress the growth of the Dunning R3327H prostate tumor for at least 30 days. The microcapsules produced a greater reduction in tumor weight than b.i.d. injections of the analog by the s.c. route in a dose that was the same or double that released from microcapsules. Testosterone levels were also depressed to a greater degree by administration of microcapsules than with the daily injections. Recently the microcapsules of [D-Trp⁶]LH-RH were also tested in rhesus monkeys and shown to delay ovulation for 30 days after a single injection as compared with controls.¶ Another agonist, [6-D-2-naphthylalanine]LH-RH administered continuously to rhesus monkeys by a microcapsule formulation reduced circulating luteinizing hormone levels (33).

[D-Trp⁶]LH-RH microcapsules permit the delivery of this peptide into the blood stream at a controlled rate over an extended period of time. This might allow the establishment of minimal blood levels necessary to achieve specified thera-

peutic effects. The use of microcapsules should eliminate large fluctuations in blood peptide levels that arise from intermittent administration. The once a month use of microcapsules will make therapy with [D-Trp⁶]LH-RH more practical and convenient and should also better ensure patient compliance. This approach should increase the utility of LH-RH analogs for contraception and treatment of prostate carcinoma, other hormone-sensitive neoplasms (34), and disorders such as precocious puberty (35) and endometriosis. Preliminary results from therapeutic trials with microcapsules of [D-Trp⁶]LH-RH in patients with prostate carcinoma|| and precocious puberty*** attest to their high efficacy.

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Table 2. Serum testosterone in rats with Dunning R3327H prostate tumors treated with microcapsules or daily injections of [D-Trp⁶]LH-RH

Treatment	Serum testosterone, ng/ml				
	Exp. 1				Exp. 2
	Day 5	Day 12	Day 19	Day 30	Day 30
Controls	2.52 ± 0.03	1.67 ± 0.21	1.01 ± 0.27	1.54 ± 0.29	1.94 ± 0.52
[D-Trp ⁶]LH-RH microcapsules, i.m.	0.14 ± 0.005*	0.15 ± 0.06*	ND	ND	ND
[D-Trp ⁶]LH-RH daily, s.c.†	0.74 ± 0.12*	0.32 ± 0.03*	0.26 ± 0.09*	0.25 ± 0.03*	0.24 ± 0.004*

Results are mean ± SEM. Significance was determined by Duncan's new multiple-range test. ND, not detectable by RIA.

*P < 0.01 compared to controls.

†Exp. 1, 12.5 µg b.i.d.; Exp. 2, 25 µg b.i.d.

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