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Inhibition of prostate tumor growth in two rat models by chronic administration of D-Trp^6 analogue of luteinizing hormone-releasing hormone

(prostate adenocarcinoma/tumor remission)

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ABSTRACT **We have investigated the effect of the D-Trp^6 analogue of luteinizing hormone-releasing hormone (LH-RH), a superactive analogue of LH-RH, on the growth of two different models of prostate tumors in rats. Chronic administration of D-Trp^6-LH-RH in a dose of 25 μg/day for 14–21 days significantly inhibited the growth of the chemically induced squamous cell carcinoma 11095 in Fisher 344 male rats. The weights of the ventral prostate and testes were also significantly reduced by treatment with this analogue. After 21 days of treatment, the animals no longer showed increases in serum luteinizing hormone and follicle-stimulating hormone levels in response to D-Trp^6-LH-RH. Treatment of male Copenhagen F-1 rats bearing the Dunning 3327 prostate adenocarcinoma with 25 μg of D-Trp^6-LH-RH per day for 42 days decreased the weights of both the ventral prostate and testes but had no effect on the weight of the anterior pituitary gland. The percentage increase in tumor volume was decreased to one-third and the actual tumor weight was decreased by 58% compared to untreated controls. The tumor doubling time was more than 4 times longer in rats receiving D-Trp^6-LH-RH than in controls. Serum levels of luteinizing hormone and follicle-stimulating hormone were significantly decreased in rats receiving this analogue. In both Fisher 344 and Copenhagen F-1 rats, serum prolactin and testosterone levels were significantly decreased after treatment with D-Trp^6-LH-RH, whereas progesterone levels were increased.**

Modification of the luteinizing hormone-releasing hormone (LH-RH) decapeptide by replacing the glycine in the 6 position by various D amino acid residues produces analogues with far greater gonadotropin-releasing activity *in vivo* and *in vitro* than the natural hormone (1–3). Among these superactive analogues is D-Trp^6-LH-RH (1), which has been tested in animals (4) as well as in humans (5, 6). Although an acute injection of superactive analogues of LH-RH causes a marked and prolonged release of gonadotropins, paradoxically, chronic administration results in chemical castration as evidenced by decreased estrogen levels and atrophy of the ovaries and uterus in female rats and by a decrease in plasma testosterone and weights of testes and accessory sex organs in male rats (7, 8). These paradoxical inhibitory effects, induced by chronic administration of superactive analogues of LH-RH, have been linked with the regression of the growth of 7,12-dimethylbenzen[*a*]anthracene-induced mammary carcinomas in the rat (9–12). In one study, an LH-RH analogue proved to be as effective as ovariectomy or tamoxifen treatment in causing regression of these tumors which were estrogen-receptor positive (11).

Lamberts *et al.* (13) showed that administration of either tamoxifen or a potent LH-RH agonist significantly inhibited the growth of transplantable prolactin-secreting rat pituitary tumors, but by different mechanisms. They suggested that tamoxifen acts by blocking estrogen receptors of the tumor, whereas the LH-RH agonist induces a chemical castration. These studies (9–13) indicate that chronic administration of large doses of LH-RH agonists may inhibit the growth of steroid-dependent tumors by suppressing both pituitary and gonadal functions.

The present study compares the effect of chronic administration of D-Trp^6-LH-RH on the growth of two distinct hormone-sensitive prostate tumors in the rat. Some of our findings have appeared in abstract form (14).

MATERIALS AND METHODS

Male Fisher 344 rats with body weights between 100 and 120 g were inoculated subcutaneously in the scapular region with cubes (2–3 mm^3) of squamous cell prostate tumor 11095 obtained from A. Segaloff (15) (Ochsner Foundation Hospital, New Orleans). D-Trp^6-LH-RH was synthesized by solid-phase methods and purified as described (1) or by classical synthesis and supplied by Ayerst Laboratories (New York). In the first two experiments, the analogue was administered subcutaneously once a day in a dose of 25 μg in 200 μl of saline for 14–21 days. Control rats bearing the 11095 tumor received injections of saline.

Male (Copenhagen × Fisher)F_1 rats bearing the androgen-dependent well-differentiated R-3327 Dunning rat adenocarcinoma were provided by Norman Altman (Papanicolaou Cancer Research Institute, Miami, FL). Tumors were measured weekly with microcalipers in all animals. All three diameters of the mass were determined and the tumor volume was calculated according to the formula L × W × H × 0.5236 as described by Janek *et al.* (16). Cell doubling time was calculated by the formula:

$$\frac{\text{days of treatment}}{[\log(\text{final vol}) - \log(\text{initial vol})]/\log 2}$$

as described by Smolev *et al.* (17). One hundred forty days after transplantation, the tumors were palpable, and rats bearing tumors 25 mm^3 or greater were selected for study. In this study, D-Trp^6-LH-RH was dissolved in a 10% polyvinylpyrrolidone in saline and injected subcutaneously once a day in a dose of 25 μg in a volume of 200 μl for 42 days. Control rats with tumors were injected with a 10% polyvinylpyrrolidone.

Rats were housed five or six in a cage in a temperature-controlled room with 12 hr light/12 hr dark schedule and were fed

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Abbreviations: LH-RH, luteinizing hormone-releasing hormone; FSH, follicle-stimulating hormone.

Table 1. Effect of chronic administration of D-Trp^6-LH-RH on body, organ, and tumor weights in male rats bearing 11095 squamous cell prostate tumors

Treatment	n	Body wt, g	Anterior pituitary, mg	Ventral prostate, mg	Testes, g	Prostate tumor, g
			Study 1 (14 days)			
Control	8	169 ± 5	4.96 ± 0.28	78.6 ± 5	2.21 ± 0.06	5.4 ± 0.8
D-Trp^6-LH-RH	10*	171 ± 4	5.82 ± 0.17	57.8 ± 3	1.01 ± 0.05	2.9 ± 0.5
P		NS	NS	<0.005	<0.001	<0.025
			Study 2 (21 days)			
Control	8	203 ± 15	5.16 ± 0.36	ND	2.28 ± 0.17	19.2 ± 2
D-Trp^6-LH-RH	8	201 ± 11	5.27 ± 0.27	ND	1.43 ± 0.10	12.0 ± 1
P		NS	NS		0.001	<0.01

NS, not significant; ND, not determined.

water and rat chow ad lib. They were sacrificed by decapitation 24 hr after the last injection, and trunk blood was collected. The blood was centrifuged and serum was collected. Various organs were removed, cleaned, and carefully weighed. Tumors were cleaned of any adhering tissue, weighed, and then quickly frozen on dry ice for chemical analyses. Some tumor tissue was processed for histological evaluation.

Serum levels of LH, follicle-stimulating hormone (FSH) and prolactin were measured by double-antibody radioimmunoassays using materials supplied by the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases (18, 19). The results are expressed in terms of ng of Rat-Prl-RP-1, Rat-LH-RP1, and Rat-FSH-RP-1 standards per ml. Serum testosterone and progesterone were measured by kits obtained from Upjohn (Kalamazoo, MI).

All data are expressed as the mean ± SEM. Statistical evaluation of tumor growth and weight and organ and body weights were made by using the Student *t* test (20).

RESULTS

The effects of chronic treatment with D-Trp^6-LH-RH on body organ and tumor weights are shown in Table 1. Neither body weight nor anterior pituitary weight was significantly changed by chronic administration of the analogue to rats bearing the 11095 squamous cell prostate tumor. The ventral prostate weight in experiment 1 was decreased by about 25% and showed a significant difference compared to control values. Testicular weights were greatly decreased in both experiments.

Prostate tumor weights were recorded after careful removal of the cystic fluid and loose dead tissue remaining inside the tumor. There was a significant decrease in tumor weight in both experiments after administration of 25 μg of D-Trp^6-LH-RH per day for either 14 or 21 days (Table 1).

After 14 days of treatment (Exp. 1), the anterior pituitaries still responded to D-Trp^6-LH-RH as evidenced by a significant increase in plasma LH and FSH levels 24 hr after the injection (Table 2). Serum testosterone levels were reduced significantly, by 34%, compared to control values. However, after injection of the analogue for 21 days (Exp. 2), the rats no longer showed increases in serum LH or FSH levels in response to the analogue and prolactin levels were decreased by 47% ($P < 0.01$) compared to control values. Serum testosterone levels in rats treated with D-Trp^6-LH-RH were decreased by >70% compared to those of controls, whereas progesterone levels were still increased by 260% 24 hr after the last injection of the analogue.

Rats bearing the hormone-sensitive Dunning R-3327 prostate tumor showed highly significant reductions in weights of both the ventral prostate and testes (Table 3) after treatment with D-Trp^6-LH-RH for 42 days, but the weight of the anterior pituitary gland remained unchanged. Body weights of rats treated with D-Trp^6-LH-RH were slightly decreased. However, these rats were somewhat smaller at the beginning of the experiment, and previous and subsequent repeated experiments with this analogue have failed to show any significant effect on body weight. In this study with slowly growing R-3327 prostate tumors there was a highly significant 72% decrease in tumor volume after 42 days of treatment with D-Trp^6-LH-RH (Table 4). The percentage increase in tumor volume was decreased to 30% of the control and tumor weight, at autopsy, was decreased by 58% in rats treated with the analogue. In rats treated with D-Trp^6-LH-RH for 42 days, mean tumor cell doubling time was 87 days compared to 18 days for tumors in control rats, but this difference was not significant statistically because of large individual variations.

Table 5 shows serum levels of some pituitary and steroid hormones in these rats. Treatment with D-Trp^6-LH-RH for 42

Table 2. Hormone levels in serum of male rats bearing 11095 squamous cell prostate tumor after chronic administration of D-Trp^6-LH-RH

Treatment	n	LH, ng/ml	FSH, ng/ml	Prolactin, ng/ml	Testosterone, ng/ml	Progesterone, ng/ml
			Study 1 (14 days)			
Control	8	12.9 ± 1.1	316 ± 6	ND	7.81 ± 0.40	ND
D-Trp^6-LH-RH	10	69.7 ± 6.0	464 ± 46	ND	5.13 ± 0.80	ND
P		<0.001	<0.01		<0.025	
			Study 2 (21 days)			
Control	8	17.0 ± 4.0	372 ± 19	52.3 ± 7.0	2.83 ± 0.36	8.2 ± 1.0
D-Trp^6-LH-RH	8	19.9 ± 4.0	364 ± 32	27.6 ± 4.0	0.80 ± 0.22	21.8 ± 9.2
P		NS	NS	<0.01	<0.001	NS

ND, not determined; NS, not significant.

Table 3. Body and organ weights in male rats bearing Dunning R-3327 prostate tumors after treatment with D-Trp^6-LH-RH for 42 days

Treatment	n	Body wt, g	Anterior pituitary, mg	Ventral prostate, mg	Testes, g
Controls	6	396 ± 9	8.75 ± 0.46	274 ± 56	3.06 ± 0.1
D-Trp^6-LH-RH	3	357 ± 12	8.36 ± 0.47	111 ± 5	1.78 ± 0.17
P		<0.05	NS	<0.025	<0.001

NS, not significant.

days significantly decreased serum levels of LH, FSH, and prolactin in rats. Serum levels of testosterone were decreased by >200%; progesterone levels were significantly increased compared to controls.

DISCUSSION

The present study demonstrates the effects of chronic administration of D-Trp^6-LH-RH, a superactive analogue of LH-RH, on the growth of two different transplantable prostate tumors. The squamous cell carcinoma was originally induced by Segaloff (15) by implanting crystals of methylcholanthrene into the ventral prostate of Fisher 344 male rats. Although this tumor may not represent an ideal model for the study of human prostatic cancer, its availability permitted us to use it for the initial examination of effects of D-Trp^6-LH-RH. Segaloff reported (15) that this tumor was hormone-sensitive and showed a decreased growth pattern in young male rats treated with testosterone and an increased growth in castrated males. He was able to show that corticoids such as dexamethasone and 11-keto-6α-methylprogesterone were also capable of inhibiting the growth of this tumor (15). Later, Bogden and Esber (21) reported that diethylstilbestrol also inhibited the growth of this tumor. It is interesting that chronic administration of D-Trp^6-LH-RH, a substance that lowers plasma testosterone and estrogen levels, had such a pronounced suppressive effect on tumor weight. This also suggests that the inhibition of growth of this tumor by D-Trp^6-LH-RH may be mediated by more than one mechanism, in accord with our findings in the Dunning prostate adenocarcinoma.

The spontaneous prostate adenocarcinoma designated R-3327 was first described by Dunning in 1963 (22). Histologically, this slowly growing well-differentiated adenocarcinoma appears to be almost identical to the human prostate adenocarcinoma (23, 24). Biochemically, the enzyme profile of this tumor is similar to that of the dorsal prostate of the rat and has a moderate 5α-reductase level (23–25). Cytoplasmic receptors of both estrogen and androgen are present in this tumor, which requires androgens for maximal growth (26–28). These similarities to human prostate adenocarcinoma have made the Dunning R-3327 tumor an acceptable animal model for the study of human prostatic cancer. The suppressive effect of D-Trp^6-LH-RH on this tumor might be explained in part by a reduction in serum testosterone levels.

The principal mechanism for the regression of both types of tumors indeed may be linked with the effect of agonists of LH-RH on the levels of sex steroids. Burgus *et al.* (29) were able to show a direct steroidogenic defect in incubated Leydig cells of rats treated with a LH-RH superagonist closely related to our analogue. In their studies, progesterone continued to be synthesized and secreted but testosterone was progressively suppressed (29). Furthermore, chronic administration, to intact male rats, of this LH-RH superagonist which differs from our analogue only by an ethylamide group in position 10, dramatically increased plasma progesterone levels and decreased testosterone levels (30). It is possible that the high levels of progesterone obtained under our experimental conditions significantly inhibited the growth of the 11095 tumor because Segaloff originally reported (15) that this tumor could be inhibited by the administration of 11-keto-6α-methylprogesterone. The effect of this steroid on the growth of the Dunning R-3327 tumor has not been studied by us. Our findings of increased plasma progesterone and decreased testosterone levels in both the Fisher 344 rats bearing 11095 prostate tumors and (Copenhagen × Fisher) F_1 rats with Dunning R-3327 adenocarcinoma after treatment with D-Trp^6-LH-RH are in agreement with the observations of Rivier and Vale (30) in normal male Sprague–Dawley rats.

Hypophysectomy, alone or in combination with orchidectomy, is the most effective means of suppressing tumor growth in the Dunning animal model (31). The resulting loss of gonadotropins and corticotropin eliminates androgen production by both the testes and the adrenals. Chronic treatment with D-Trp^6-LH-RH can partially mimic hypophysectomy by decreasing plasma levels of gonadotropins, prolactin, and testosterone. Prolactin has been shown to have a stimulatory effect on prostate growth without mediation through the adrenals or the testes (32). In combination with testosterone, prolactin has been shown to have synergistic effects on prostate growth (32). The decreased plasma levels of prolactin and testosterone in rats bearing the Dunning R-3327 prostate tumor and treated with D-Trp^6-LH-RH would be expected to suppress tumor growth.

Another explanation of the inhibitory action of D-Trp^6-LH-RH on prostate tumor growth may be a direct antagonism by this substance of the effects of sex steroids. Sundaram *et al.* (33) reported that D-Trp^6-LH-RH ethylamide could reduce accessory sex organ weight in hypophysectomized male rats maintained on moderate doses of testosterone and suggested that this superagonist could antagonize the action of sex steroids.

In addition, it is also possible that D-Trp^6-LH-RH might have a direct inhibitory effect on prostate tissue. LH-RH receptors have been found in the testes and ovaries, but there is a paucity of data on these receptors in accessory sex organs. The presence of LH-RH receptors in prostate tissue could offer an additional explanation for the effects of agonists of LH-RH on the growth of prostate tumors because it has been shown that such analogues can decrease LH-RH receptors in the testes (34).

The finding that D-Trp^6-LH-RH inhibits the growth of prostate tumor in rats could have a potential clinical application.

Table 4. Response of Dunning R-3327 prostate tumor to treatment with D-Trp^6-LH-RH for 42 days

Treatment	n	Tumor volume, mm^3 Initial	Tumor volume, mm^3 Final	% increase	Tumor wt, mg	Cell doubling time, days
Control	6	109 ± 28	633 ± 155	639 ± 115	797 ± 173	18 ± 3
D-Trp^6-LH-RH	5	126 ± 51	174 ± 42	189 ± 68	322 ± 71	87.3 ± 41
P		NS	<0.025	<0.01	<0.05	NS

NS, not significant.

Table 5. Serum hormone levels in male rats bearing Dunning R-3327 prostate tumor after chronic treatment with D-Trp^6-LH-RH

Treatment	n	LH, ng/ml	FSH, ng/ml	Prolactin, ng/ml	Testosterone, ng/ml	Progesterone, ng/ml
Controls	5	38.5 ± 5	2044 ± 412	178 ± 8	3.42 ± 0.72	6.65 ± 1.39
D-Trp^6-LH-RH	5	0.00 ± 0	760 ± 44	92.6 ± 9	0.81 ± 0.26	16.7 ± 1.7
P		<0.001	<0.025	0.001	0.01	0.005

Tolis *et al.* (unpublished data; *) have reported a clinical improvement and reduction in tumor size in patients with prostate carcinoma after treatment with D-Trp^6-LH-RH. The analogue of LH-RH may thus lead to development of a new type of therapy for prostate carcinoma and other hormone-dependent tumors in man.

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* Tolis, G., Mehta, A., Dimitrakpoulos, C., Stellos, A., Ackman, D., Kinch, R., Comaru-Schally, A. M. & Schally, A. V., Annual Meeting of the Endocrine Society, Cincinnati, OH, June 1981, p. 21 (abstr. 33).

1. Coy, D. H., Vilchez-Martinez, J. A., Coy, E. J. & Schally, A. V. (1976) *J. Med. Chem.* **119**, 423–425.
2. Schally, A. V., Coy, D. H. & Arimura, A. (1980) *Int. J. Gynaecol. Obstet.* **18**, 318–324.
3. Schally, A. V., Arimura, A. & Coy, D. H. (1980) *Vitam. Horm.* **38**, 257–323.
4. Vilchez-Martinez, J. A., Pedroza, E., Arimura, A. & Schally, A. V. (1979) *Fertil. Steril.* **31**, 677–682.
5. Jaramillo-Jaramillo, C., Charro-Salgado, A., Perez-Infante, V., Lopez del Campo, G., Botella-Llusia, J., Coy, D. H. & Schally, A. V. (1978) *Fertil. Steril.* **29**, 418–423.
6. Wass, J. A. H., Besser, G. M., Gomez-Pan, A., Scanlon, M. F., Hall, R., Kastin, A. J., Coy, D. H. & Schally, A. V. (1979) *Clin. Endocrinol.* **10**, 419–430.
7. Johnson, E. S., Gendrich, R. L. & White, W. F. (1976) *Fertil. Steril.* **27**, 853–860.
8. Rivier, C., Rivier, J. & Vale, W. (1979) *Endocrinology* **105**, 1191–1201.
9. DeSombre, E. R., Johnson, E. S. & White, W. F. (1976) *Cancer Res.* **36**, 3830–3833.
10. Danguy, A., Legros, N., Henson-Stiennon, J. A., Pasteels, J. H., Atassi, G. & Heuson, J. C. (1977) *Eur. J. Cancer* **13**, 1089–1094.
11. Nicholson, R. I. & Maynard, P. V. (1979) *Br. J. Cancer* **39**, 268–273.
12. Rose, D. P. & Pruitt, B. (1979) *Cancer Res.* **39**, 3968–3970.
13. Lamberts, S. W. J., Uitterlinden, P., Zuiderwizk-Roest, J. M., Bons-Van Evelingen, E. G. & deJong, F. H. (1981) *Endocrinology* **108**, 1878–1884.
14. Redding, T. W. & Schally, A. V. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 433 (abstr.).
15. Segaloff, A. (1962) *Natl. Cancer Inst. Monogr.* **12**, 407–408.
16. Janek, P., Briand, P. & Hartmann, N. R. (1975) *Cancer Res.* **35**, 3698–3704.
17. Smolev, J. K., Heston, W. D. W., Scott, W. W. & Coffey, D. S. (1977) *Cancer Treat. Rep.* **61**, 273–287.
18. Davis, S. L., Reichert, L. E., Jr. & Niswender, G. D. (1971) *Biol. Reprod.* **4**, 145–151.
19. Daana, T. A. & Parlow, A. F. (1971) *Endocrinology* **88**, 653.
20. Snedecor, G. W. (1961) *Statistical Methods* (Iowa State Univ. Press, Ames, IA).
21. Bogden, A. E. & Esber, H. J. (1976) *Natl. Cancer Inst. Mongr.* **49**, 263–267.
22. Dunning, W. F. (1963) *Natl. Cancer Inst. Mongr.* **12**, 351–370.
23. Smolev, J. K., Coffey, D. S. & Scott, W. W. (1977) *J. Urol.* **118**, 216–220.
24. Lubaroff, D. M., Canfield, L., Rasemussen, G. T. & Reynolds, G. W. (1976) *Natl. Cancer Inst. Monogr.* **49**, 275–281.
25. Voigt, W. & Dunning, W. F. (1974) *Cancer Res.* **34**, 1447–1450.
26. Markland, F. & Lee, L. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 913.
27. Markland, F. & Lee, L. (1979) *J. Steroid Biochem.* **10**, 13–20.
28. Heston, W. D. W., Menon, M., Tananis, C. & Walstt, P. C. (1979) *Cancer Lett.* **6**, 45–50.
29. Burgus, R., Butcher, M., Amoss, M., Ling, N., Monohan, M., Rivier, J., Fellows, R., Blackwell, R., Vale, W. & Guillemin, R. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 278–282.
30. Rivier, C. & Vale, W. (1979) *Life Sci.* **25**, 1065–1074.
31. Shessel, F. S., Block, N. L., Stoner, B., Claflin, A., Malenin, T. I. & Politana, V. A. (1980) *Invest. Urol.* **17**, 529–533.
32. Grayhack, J. T. (1962) *Natl. Cancer Inst. Monogr.* **12**, 189–199.
33. Sundaram, K., Cao., Y. Q., Wang, N., Bardin, C. W., Rivier, S. & Vale, W. (1981) *Life Sci.* **28**, 83–88.
34. Sharpe, R. M. & Fraser, H. M. (1980) *Biochem. Biophys. Res. Commun.* **95**, 256–262.