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Prostate carcinoma tumor size in rats decreases after administration of antagonists of luteinizing hormone-releasing hormone

(prostate adenocarcinoma/inhibitory analogues of luteinizing hormone-releasing hormone/tumor remission)

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ABSTRACT **The effects of two potent antagonistic analogues of luteinizing hormone-releasing hormone (LH-RH) on the growth of two different models of rat prostate tumors have been investigated. Chronic administration of [NAc-p-F-DPhe^1,p-Cl-DPhe^2,DTrp^{3,6},D-Ala^{10}]LH-RH (antagonist I) at 50 μg/day for 21 days significantly inhibited the growth of the chemically induced squamous cell carcinoma 11095 in Fisher 344 male rats. The weights of the pituitary, ventral prostate, and testes were not significantly altered. After 21 days of treatment with this analogue serum luteinizing hormone (lutropin), follicle-stimulating hormone, and testosterone levels were markedly decreased. When male Copenhagen F-1 rats bearing the Dunning 3327H prostate adenocarcinoma were injected with antagonist I at 50 μg/day for 6 weeks or with [NAc-p-Cl-DPhe^{1,2},DTrp^3,DPhe^6,DAla^{10}]LH-RH (antagonist II) at 50 μg/day for 17 days, the percentage increase in tumor volume was decreased to half or less and the actual tumor volume was diminished 34–96% compared to controls. Tumor weight was decreased 30% and 89% after antagonist I and II, respectively, compared to untreated controls. The tumor doubling time was 3- to 4-fold longer in rats receiving the inhibitory analogues than in the controls. Treatment with antagonist II decreased the weight of the whole prostate, but neither antagonist changed the weight of testes, anterior pituitary gland, or adrenals. Serum luteinizing hormone, follicle-stimulating hormone, and testosterone levels in Copenhagen F-1 rats bearing Dunning tumors were significantly decreased after treatment with the inhibitory analogues, but progesterone levels were increased. The inhibitory effects of these antagonistic analogues on rat prostrate tumors suggest that these compounds might be considered in the development of new types of therapy for prostate carcinoma and other endocrine-dependent neoplasias.**

Certain modifications of the decapeptide luteinizing hormone-releasing hormone (LH-RH) by NH_2-terminal alterations and further substitutions with some D amino acids in positions 2, 3, and 6—and in some instances other changes—produce inhibitory analogues which may act on the same receptor sites as LH-RH, but which decrease instead of stimulating the release of gonadotropins (1–3). A series of potent antagonists of LH-RH has been synthesized and tested in a number of animal species (4–7) and in humans (8–11). These antagonists inhibit LH-RH-induced LH and follicle-stimulating hormone (FSH) release and block ovulation and the preovulatory surge of gonadotropins in rats, hamsters, and rabbits (1–7). In women and in subhuman primates, the inhibitory LH-RH analogues disrupt normal events in the menstrual cycle, block ovulation, and inhibit the increase in LH and FSH induced by oophorectomy (9–13). Administration of large doses of LH-RH antagonists has also been reported to abolish mating behavior, decrease the testosterone production, decrease the weights of testes and accessory sex organs, and disrupt spermatogenesis in male rats (7, 14). By 4–8 weeks after cessation of treatment, mating behavior and fertility were restored (7, 14).

Chronic administration of superactive agonistic analogues of LH-RH can result in a paradoxical inhibition of the pituitary–gonadal axis characterized by the atrophy of gonads and accessory sex organs and a decrease in the levels of sex steroids (1–3). We have recently demonstrated that prolonged treatment with [DTrp^6]LH-RH, a superagonist of LH-RH, inhibits the growth of prostate tumors in two rat models (15). Because antagonists of LH-RH can decrease serum testosterone levels and weights of androgen-dependent organs, these substances might also have a potential application in the control of growth of steroid-dependent tumors. In the study reported here we assessed the effect of chronic administration of potent antagonists of LH-RH to rats bearing two different models of prostate tumors.

MATERIALS AND METHODS

Fisher 344 male rats (body weight, 100–200 g) were inoculated subcutaneously in the scapular region with 2- to 3-mm^3 cubes of squamous cell prostate carcinoma 11095 (16), kindly supplied by A. Segaloff (Ochsner Foundation Hospital, New Orleans, LA). Antagonistic analogue I, [NAc-p-F-DPhe^1,p-Cl-DPhe^2,DTrp^{3,6},DAla^{10}]LH-RH, was synthesized in our laboratory as described (5). The peptide was dissolved in 40% propylene glycol in saline to a concentration of 250 μg/ml and administered subcutaneously once a day at a dose of 50 μg for 21 days. Control tumor-bearing rats received injections of 200 μl of 40% propylene glycol in saline.

(Male Copenhagen × Fisher) F_1 rats bearing the androgen-dependent well-differentiated R-3327H Dunning rat adenocarcinoma were kindly provided by Norman Altman (Papanicolaou Cancer Research Institute, Miami, FL). Tumors were palpable 140 days after transplantation, and rats bearing tumors 25 mm^3 or greater were selected for the study. Two different antagonists were used in this tumor model. The first was analogue I. The second was analogue II, [NAc-p-Cl-DPhe^{1,2},DTrp^3,DPhe^6,DAla^{10}]LH-RH, prepared by solid-phase methods in our laboratory and purified as described (5) or by classical synthesis and supplied by Organon N.V. Oss, Netherlands (batch LO-1037B). Antagonists I and II were dissolved in 40% propylene glycol as above and injected subcutaneously once a day at a dose of 50

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Abbreviations: LH-RH, luteinizing hormone-releasing hormone; LH, luteinizing hormone (lutropin); FSH, follitropin (follicle-stimulating hormone).

Table 1. Effect of chronic administration* of LH-RH antagonist I in male rats bearing Segaloff 11095 tumor

Treatment	Weights†				
	Body, g	Anterior pituitary, mg	Testes, g	Ventral prostate, mg	Prostate tumor, g
Controls (n = 8)	169 ± 5	4.96 ± 0.28	2.21 ± 0.06	78.6 ± 5	5.37 ± 0.83
Antagonist I (n = 10)	160 ± 7	4.83 ± 0.23	1.99 ± 0.10	77.6 ± 7	3.04 ± 0.69
P	NS	NS	NS	NS	<0.05

* At 50 μg/day for 21 days.
† All results are shown as mean ± SEM.

μg for 6 weeks or 17 days, respectively. Control rats were injected with the vehicle.

Rats were housed five or six to a cage in a temperature-controlled room with a 12-hr light/12-hr dark schedule and fed water and rat chow ad lib. Tumors were measured with calipers, and tumor volume and tumor doubling time were calculated as described (15). Rats were sacrificed by decapitation 1–2 hr after the last injection of peptide. Trunk blood was collected and serum was separated for further analyses. Various organs were removed, cleaned, carefully weighed, and quickly frozen on dry ice for further chemical analyses. Some tumor tissues were processed for histological evaluation.

Plasma levels of LH, FSH, and prolactin were measured by a double-antibody radioimmunoassay using materials supplied by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases Rat Pituitary Hormone Distribution Program as described (15). Serum testosterone and progesterone were measured by using radioimmunoassay kits from Upjohn. The percentage increase in tumor volume was calculated on the basis of individual increases. All data are expressed as the mean ± SEM. Statistical analyses were performed by using the Student t test (17).

RESULTS

Table 1 shows the effect of chronic administration (50 μg/day for 21 days) of LH-RH antagonist I on body, organ, and tumor weights in rats bearing the Segaloff 11095 tumor. Body and anterior pituitary weights were not changed, nor were the weights of the ventral prostate or testes altered significantly. However, there was a 43% decrease in tumor weight ($P < 0.05$). There was also a highly significant decrease in the serum levels of both LH and FSH after treatment with the antagonist (Table 2). Similarly, serum testosterone levels were greatly decreased compared to controls.

Administration of LH-RH antagonist I for 6 weeks or antagonist II for 17 days to rats bearing the Dunning adenocarcinoma R-3327H did not significantly affect body, anterior pituitary, or testes weights (Table 3). The ventral prostate was not changed after prolonged administration of antagonist I. However, the whole prostate gland showed a highly significant decrease in weight in response to antagonist II. The percentage increase in tumor volume was reduced 50% by analogue I. Tumor volume decreased in response to antagonist II. Tumor volume was smaller in the groups treated with the inhibitory analogues. Antagonist II decreased it to 4% of controls (Table 4). Tumor weights were decreased by 30% with antagonist I and 89% with antagonist II compared to control rats; only the latter difference was statistically significant. In one rat treated with analogue II, the tumor disappeared completely. Calculation of the tumor doubling time also confirmed the reduction in growth of the Dunning prostate tumors in rats treated with the antagonists. The tumor doubling time was 3 and 4 times longer in the groups treated with antagonists I and II, respectively, compared to the control rats.

Table 2. Effect of chronic administration* of LH-RH antagonist I on serum hormone levels in rats bearing Segaloff 11095 tumor

Treatment	Serum level, ng/ml†		
	LH	FSH	Testosterone
Controls (n = 8)	12.9 ± 1.1	316 ± 6	7.81 ± 0.41
Antagonist I (n = 10)	4.1 ± 1.0	224 ± 8	1.07 ± 0.23
P	<0.001	<0.001	0.001

* At 50 μg/day for 21 days.
† All results are shown as mean ± SEM.

Long-term administration of antagonist I or II to (Copenhagen × Fisher)F_1 rats bearing Dunning prostate tumors resulted in a significant decrease in serum LH and FSH levels compared to controls treated with 40% propylene glycol (Table 5). Prolactin levels were significantly decreased with antagonist I. Testosterone levels were decreased by more than 50% with both antagonists, but progesterone levels were significantly increased.

DISCUSSION

Segaloff (16) reported that the chemically induced squamous cell prostate carcinoma 11095 was hormone-sensitive because its weight decreased after the administration of testosterone, dexamethasone, or 11-keto-6α-methylprogesterone. Bogden and Esber (18) were also able to show that diethylstilbestrol inhibited the growth of this tumor. This suggests, in accord with our previous findings with [DTrp^6]LH-RH (15), that several mechanisms may exist whereby the antagonists and agonists of LH-RH may inhibit the growth of prostate tumors.

The androgen-dependency of the Dunning prostate adenocarcinoma R-3327H is well documented (19–21). From 70% to 90% of the cells of this tumor appear to be dependent on androgen for maximal growth stimulation. The marked decrease in testosterone levels after chronic administration of 50 μg of antagonist I or antagonist II for 42 or 17 days, respectively, results in testosterone deprivation of tumor cells and could account for the reduction in tumor growth. However, under our experimental conditions, this decrease in testosterone levels did not lead to a reduction in the weights of testes. Rivier *et al.* (7, 14) obtained a decrease in the weights of testes after daily administration of 1-mg doses of their antagonist [Ac-Δ^3-Pro^1,*p*-Cl-DPhe^2,DTrp^{3,6},N^{α}-MeLeu^7]LH-RH. This difference between results could be explained by their use of doses 20 times larger than ours, although the analogues tested by us may be more potent than theirs. A greater inhibition of testicular and ventral prostate weights in our previous investigation (15) with the agonist [DTrp^6]LH-RH could suggest that superactive an-

Table 3. Effect of chronic administration of LH-RH antagonists in rats bearing Dunning prostate tumor R-3327H

Treatment	Weights*					
	Body, g	Anterior pituitary, mg	Adrenals, mg	Testes, g	Ventral prostate, mg	Whole prostate, mg
Controls (n = 6)	393 ± 9	8.75 ± 0.46	ND	3.06 ± 0.10	274 ± 56	ND
Antagonist I (n = 5)†	409 ± 8	9.96 ± 0.42	ND	3.11 ± 0.04	243 ± 22	ND
P	NS	NS		NS	NS	
Controls (n = 5)	440 ± 38	10.09 ± 0.90	63.5 ± 6	3.22 ± 0.15	ND	728 ± 57
Antagonist II (n = 4)‡	453 ± 18	11.19 ± 0.30	60.8 ± 8	3.32 ± 0.07	ND	448 ± 17
P	NS	NS	NS	NS		<0.005

* Mean ± SEM. ND, not determined.
† At 50 μg/day for 6 weeks.
‡ At 50 μg/day for 17 days.

alogues of LH-RH exert a suppressive effect directly on the testes and that they could antagonize the effects of testosterone (22).

The principal mechanism for the inhibition of growth of both the 11095 squamous cell carcinoma and the Dunning R-3327H adenocarcinoma is likely to be linked with the effect of LH-RH antagonists on the levels of sex steroids. Some superagonists of LH-RH have been shown to decrease plasma testosterone and raise progesterone levels significantly in male rats (15, 23). Plasma progesterone levels were also increased in the present study in the Dunning rat model after chronic administration of the antagonistic analogues. It is possible that high levels of progesterone obtained under our experimental conditions contributed to the inhibition of the growth of the prostate tumors. Segaloff originally reported that the growth of the tumor 11095 was inhibited by the administration of 11-keto-6α-methylprogesterone (16).

The histological and biochemical similarities of the Dunning R-3327 prostate tumor to human prostate adenocarcinoma have made this tumor an acceptable model for the study of human prostate cancer (24–26). The most effective means of suppressing tumor growth in the Dunning rat model is hypophysectomy, alone or in combination with orchidectomy (27). The resulting loss of gonadotropins and corticotropin abolishes androgen production by the testes and the adrenals. The suppressive effect of the LH-RH antagonists on gonadotropins, prolactin, and testosterone therefore can mimic in part the effect of hypophysectomy. Prolactin alone or in combination with testosterone has been shown to have a stimulatory effect on prostate growth without mediation through the adrenals and testes (28). Suppression of both testosterone and prolactin by chronic administration of LH-RH antagonists might thus be expected to inhibit tumor growth more effectively than the deficiency of androgen alone.

It is also possible that LH-RH antagonists could have some direct inhibitory effect on the prostate tumors. LH-RH receptors have been found in the rat testes and ovaries, but there is no information on the levels of these receptors in accessory sex organs. The presence of LH-RH receptors in prostate tissue or tumor tissue could be an additional explanation for the effects of the antagonist on the growth of the prostate tumors. We have previously shown that some LH-RH antagonists bind to pituitary receptors in a competitive fashion and that even single low doses decrease pituitary binding sites for LH-RH (29, 30). It would not be surprising if this should occur in prostate tissue, if receptors do indeed exist there.

The feasibility of use of inhibitory analogues of LH-RH for contraception was proved by our clinical work (8–11). We have previously reported that [DTrp^6]LH-RH, a superagonist of LH-RH, suppressed the growth of prostate tumor in rats (15) and men (31, 32). The inhibitory effects of antagonistic analogues of LH-RH on rat prostate tumors suggest that these compounds might also be considered for the development of new types of therapy for prostate carcinoma and other endocrine-dependent neoplasias.

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Table 4. Response of Dunning tumor R-3327H to treatment with inhibitory analogs of LH-RH

Treatment	Tumor volume			Tumor weight, mg	Tumor doubling time, days
	Initial, mm^3	Final, mm^3	% increase		
Controls (n = 6)	109 ± 28	633 ± 155	639 ± 115	797 ± 173	15 ± 13
Antagonist I (n = 5)	205 ± 91	419 ± 140	286 ± 29	554 ± 196	48 ± 17
P	NS	NS	<0.025	NS	NS
Controls (n = 5)	698 ± 110	1375 ± 324	188 ± 24	1390 ± 440	28 ± 7
Antagonist II (n = 4)	1632 ± 950	57 ± 42	−82 ± 12	158 ± 120	122 ± 40
P	NS	<0.005	<0.001	<0.05	NS

All results are shown as mean ± SEM. Treatments were as in Table 3.

Table 5. Effect of chronic administration of LH-RH antagonists on serum hormone levels in rats bearing Dunning prostate tumor R-3327H

Treatment	Serum level, ng/ml				
	LH	FSH	Prolactin	Testosterone	Progesterone
Controls (n = 6)	39 ± 5	2044 ± 412	178 ± 8	3.42 ± 0.72	6.65 ± 1.4
Antagonist I (n = 5)	18 ± 7	1080 ± 75	107 ± 3	1.57 ± 0.34	18.75 ± 4.8
P	<0.05	<0.05	<0.001	0.05	0.05
Controls (n = 5)	199 ± 14	3455 ± 327	48.2 ± 4.9	9.42 ± 0.53	51.2 ± 8
Antagonist II (n = 4)	142 ± 10	2169 ± 288	35.6 ± 4.6	3.89 ± 0.30	159.5 ± 18
P	<0.025	<0.025	NS	<0.001	0.001

All results are shown as mean ± SEM. Treatments were as in Table 3.

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