Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 844–848, February 1991
Medical Sciences

Inhibition of growth of experimental prostate cancer with sustained delivery systems (microcapsules and microgranules) of the luteinizing hormone-releasing hormone antagonist SB-75

(luteinizing hormone-releasing hormone analogs/chemical castration/hormone-sensitive cancers)

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Contributed by A. V. Schally, October 26, 1990

ABSTRACT **Inhibitory effects of the sustained delivery systems (microcapsules and microgranules) of a potent antagonist of luteinizing hormone-releasing hormone *N*-Ac-[3-(2-naphthyl)-D-alanine[1], 4-chloro-D-phenylalanine[2], 3-(3-pyridyl)-D-alanine[3], D-citrulline[6], D-alanine[10]]LH-RH (SB-75) on the growth of experimental prostate cancers were investigated. In the first experiment, three doses of a microcapsule preparation releasing 23.8, 47.6, and 71.4 μg of antagonist SB-75 per day were compared with microcapsules of agonist [D-Trp[6]]LH-RH liberating 25 μg/day in rats bearing Dunning R3327H transplantable prostate carcinoma. During 8 weeks of treatment, tumor growth was decreased by [D-Trp[6]]LH-RH and all three doses of SB-75 as compared to untreated controls. The highest dose of SB-75 (71.4 μg/day) caused a greater inhibition of prostate cancer growth than [D-Trp[6]]LH-RH as based on measurement of tumor volume and percentage change in tumor volume. Doses of 23.8 and 47.6 μg of SB-75 per day induced a partial and submaximal decrease, respectively, in tumor weight and volume. Tumor doubling time was the longest (50 days) with the high dose of SB-75 vs. 15 days for controls. The body weights were unchanged. The weights of testes, seminal vesicles, and ventral prostate were greatly reduced in all three groups that received SB-75, and testosterone levels were decreased to nondetectable values in the case of the two higher doses of SB-75. LH levels were also diminished. Similar results were obtained in the second experiment, in which the animals were treated for a period of 8 weeks with microgranules of SB-75. Therapy with microgranules of SB-75 significantly decreased tumor growth as measured by the final tumor volume, the percentage change from the initial tumor volume, and the reduction in tumor weight. The results indicate that antagonist SB-75, released from sustained delivery systems, can produce a state of chemical castration and effectively inhibit the growth of experimental prostate cancers. The efficacy of the antagonist SB-75 in inhibiting androgen-dependent Dunning prostatic carcinoma and the absence of side effects suggest its possible usefulness for the treatment of hormone-sensitive tumors.**

Although androgen deprivation represents standard treatment for prostatic cancer, new approaches are constantly explored to improve the therapy in terms of response rates, duration of response, and increase in survival rate (1, 2). Since the isolation and structural elucidation of hypothalamic luteinizing hormone-releasing hormone (LH-RH), more than 3000 analogs were synthesized in view of their potential medical applications (3, 4). The inhibition of the pituitary-gonadal axis by chronic administration of LH-RH agonistic analogs, including [D-Trp[6]]LH-RH, shown in animals (5) and man (6), led to demonstration of the suppression of growth of prostate tumors in animals (7) and their successful use in the treatment of prostatic cancer in patients (8) as a better alternative to castration or estrogens (1, 3). Although repeated administration of LH-RH agonists is required to inhibit luteinizing hormone (LH) release and reduce the levels of sex steroids, similar effects can be obtained with a single dose of LH-RH antagonists (1, 3, 4). The antagonistic analogs were synthesized initially for use in gynecology as contraceptives (1, 3). However, progress in development and clinical application of LH-RH antagonists has been slow (1). High-dose requirements, due to low potency of earlier compounds and side effects related to histamine release of analogs with D-arginine in position 6, delayed clinical use of LH-RH antagonists (1, 4, 9).

Modern LH-RH antagonists, free of edematogenic and anaphylactoid reactions, containing neutral hydrophilic D-ureidoalkyl amino acids such as D-citrulline and D-homocitrulline at position 6, were recently synthesized in our laboratory and tested *in vitro* and *in vivo* (10–12). Among these analogs, *N*-Ac-[3-(2-naphthyl)-D-alanine[1], 4-chloro-D-phenylalanine[2], 3(3-pyridyl)-D-alanine[3], D-citrulline[6], D-alanine[10]]LH-RH {[Ac-D-Nal(2)[1], D-Phe(4Cl)[2], D-Pal(3)[3], D-Cit[6], D-Ala[10]]-LH-RH; SB-75} was shown to be one of the most powerful antagonists in blocking ovulation and inhibiting LH levels in rats (9, 11, 12). The use of antagonists in tumor therapy would avoid the transient stimulation that occurs initially in response to LH-RH agonists and prevent the temporary clinical "flare-up" of the disease.

Further improvement in the convenience and reliability of treatment could be achieved by the use of long-acting delivery systems for controlled release of peptides (1, 3, 12–15). Such formulations could be injected at monthly intervals. Since 1983, we have been developing sustained-delivery systems for [D-Trp[6]]LH-RH and somatostatin analog RC-160 (13, 14). These systems are based on microcapsules or microgranules of polymers of DL-lactide-coglycolide, which are biodegradable and compatible with living tissues (12, 13, 15). We have demonstrated the efficacy of formulations of microcapsules of the agonist [D-Trp[6]]LH-RH experimentally (13, 16–18) and clinically (1, 3, 19). Prototypes of microcapsules of somatostatin analog RC-160 and, more recently, of LH-RH antagonist SB-75 were also used in the treatment of experimental breast cancer and pancreatic cancer (20, 21). More modern forms of sustained-delivery systems, which consist of microparticles (microgranules) containing SB-75 pamoate or [D-Trp[6]]LH-RH, also have been prepared (9, 12, 15). Microcapsules are more uniform spherical particles with

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Abbreviations: LH, luteinizing hormone; LH-RH, LH-releasing hormone; FSH, follicle-stimulating hormone; Cit, citrulline; Nal(2), *N*-Ac-3-(2-naphthyl)-D-alanine; Pal(3), 3-(3-pyridyl)-D-alanine; Phe(4Cl), 4-chloro-D-phenylalanine.

a diameter of about 50 μm, whereas microparticles obtained by cryogenic grinding of extruded polymer containing the homogeneously dispersed peptide are amorphous with a wide variety of sizes (9, 15).

The present study was aimed at comparing the effects of the microcapsules of [D-Trp^6]LH-RH and microcapsules and microgranules of SB-75, given at different doses, on R-3327 Dunning rat adenocarcinoma. This tumor was shown previously to be a good model of human prostatic cancer and reproducibly responsive to LH-RH agonists (7, 16–18) and older hydrophobic and hydrophilic antagonists (22, 23).

MATERIALS AND METHODS

Male (Copenhagen × Fisher)F_1 rats, bearing Dunning R-3327H transplantable prostate adenocarcinomas, were kindly provided by Norman Altman (Papanicolaou Cancer Research Institute, Miami). Tumors were measured weekly with calipers and the tumor volume was calculated as described (7, 16–18, 22). Animals were housed four in a cage in a temperature-controlled room with a 12-hr light/12-hr dark schedule and fed water and rat chow ad libitum.

Microcapsules of [D-Trp^6]LH-RH in poly(DL-lactide-coglycolide) were prepared by a phase-separation process by P. Orsolini at Cytotech (Martigny, Switzerland) and supplied by Debiopharm (Lausanne, Switzerland) (13, 16). Microcapsules of [D-Trp^6]LH-RH in aliquots of 36 mg were designed to release 25 μg/day for 30 days. Microcapsules of [D-Trp^6]-LH-RH were suspended in 0.7 ml of injection vehicle [2% (wt/vol), CM-cellulose and 1% Tween 80 in water] and injected i.m. once a month.

The LH-RH antagonist [Ac-3-(2-naphthyl)-D-Ala^1, D-Phe-(4Cl)^2, 3-(3-pyridyl)-D-Ala^3, D-Cit^6, D-Ala^{10}]LH-RH (SB-75) was synthesized by solid-phase methods in our laboratory (11). Bulk amounts of SB-75 were prepared according to the same methods by Asta Pharma (Frankfurt/Main) for microencapsulation. This product was identical with our preparation, physicochemically (HPLC, IR spectrum) and biologically *in vitro* and *in vivo* (9, 12). SB-75 microcapsules were prepared by P. Orsolini, Cytotech, using a phase separation process and sterilized by γ-irradiation. The product was a free-flowing powder of nearly spherical particles consisting of 2% (wt/wt) SB-75 trifluoroacetate in a polymeric matrix of poly(DL-lactide-coglycolide) (12). Three batches (lot nos. RCS 0003, 0004, and 0005) were used in this study.

SB-75 pamoate microgranules (prototype lot RGS 9002) were also prepared by P. Orsolini (Cytotech) as described (15) and consisted of SB-75 pamoate [4.5% (wt/wt)] distributed within a polymer matrix of poly(DL-lactide-coglycolide). For injection, the microgranules were suspended in 0.7 ml of injection vehicle consisting of 2% CM-cellulose and 1% Tween 80 in water. The suspension was mixed thoroughly on a Vortex mixer and injected i.m. through an 18-gauge needle.

In the first experiment, 19 weeks after implantation, tumors were well developed and the animals were injected i.m. into the thigh with three graded doses of microcapsules of SB-75 trifluoroacetate (25 mg, 50 mg, and 75 mg, releasing 23.8, 47.6, and 71.4 μg/day, respectively). Two more injections were given at 3-week intervals. [D-Trp^6]LH-RH (36 mg total dose, releasing 25 μg/day) was injected the first day and repeated after 4 weeks. In the second study, treatment was started 22 weeks after transplantation, when tumors were well developed, and continued for 8 weeks. [D-Trp^6]LH-RH microcapsules were injected according to the same regimen as in experiment 1. SB-75 microgranules in aliquots of 30 mg were injected every 2 weeks. This dose was estimated to release about 45–60 μg of SB-75 per day, after an initial burst effect, and to maintain a blood level of 15–45 ng of SB-75 per ml.

After 8 weeks of treatment, rats were sacrificed by decapitation and trunk blood was collected. The blood was centrifuged and serum was preserved for hormone studies. Various organs (testicles, seminal vesicles, ventral prostates) were removed, cleaned, weighed, and preserved for pathological studies, which will be reported elsewhere (24). Pituitary glands were removed and frozen at −80°C for receptor studies described elsewhere (25). Tumors were carefully cleaned and weighed, and then a sample was taken for histological studies and the rest was stored at −80°C for receptor studies (25).

Serum levels of hormones were measured by double-antibody RIAs using materials supplied by the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases, as described (12, 16). The results are expressed in terms of ng of Rat-LH-RP-3, Rat-GH-RP-2, Rat-FSH-RP-2, and Rat-Prl-RP-3 standards per ml. Serum testosterone was measured by RIA using kits obtained from Diagnostic Products, Los Angeles (9).

All data are expressed as the mean ± SEM and statistical analyses were performed by using a computer-assisted program and Duncan's new multiple-range test (26).

RESULTS

In both experiments, sustained delivery systems of LH-RH antagonist SB-75 effectively inhibited the growth of Dunning R-3327 prostate cancers. The effects of microcapsules of [D-Trp^6]LH-RH and SB-75 on tumor volume are shown in Fig. 1. Although the volume of the tumors in the control group increased continuously over a period of 8 weeks, [D-Trp^6]-LH-RH and all three doses of SB-75 markedly inhibited tumor growth. This effect became significant after 2 weeks for [D-Trp^6]LH-RH and the higher dosage of SB-75 and after 4 weeks for the lowest dose of SB-75 as compared to untreated controls. The tumor inhibition was dose dependent, higher doses of SB-75 exerting a greater suppressive effect on the growth of Dunning prostate cancers. The highest dosage of this antagonist (71.4 μg/day) suppressed tumor growth more than [D-Trp^6]LH-RH, although the difference is not significant. The results of treatment on final tumor volume, percent increase in tumor volume, and tumor doubling time are shown in Table 1. The lowest dose of SB-75 (23.8 μg/day) was effective, but the inhibition of the tumor growth, based on final volume or percentage increase in tumor volume, was less than that obtained with the agonist [D-Trp^6]LH-RH. With increasing doses of SB-75 (47.6 μg/day and, especially, 71.4 μg/day) the inhibitory effect was more pronounced and the suppression of tumor growth became comparable to or greater than that achieved with [D-Trp^6]LH-RH. Calculation of tumor doubling time also showed the reduction in tumor growth. Compared to controls, the tumor doubling time was longest with the highest dose of SB-75 (50 days).

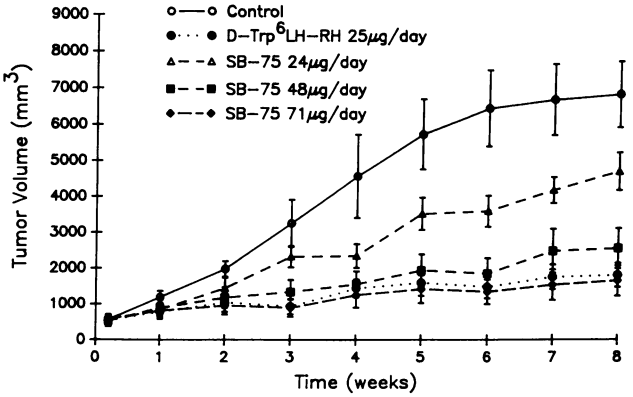

FIG. 1. Effect of treatment with microcapsules of [D-Trp^6]LH-RH or antagonist SB-75 on tumor volume in rats with Dunning R3327 prostate cancer. Vertical lines indicate the SEM.

Table 1. Effect of treatment with [D-Trp^6]LH-RH or SB-75 microcapsules on tumor volume, percent increase in tumor volume, and tumor doubling time in rats with Dunning prostate cancers

Group	Tumor volume, mm^3		% increase	Tumor doubling time, days
	Initial	Final		
Control	554.5 ± 102.6	6815.5 ± 908.1	1273.8 ± 157.2	15 ± 1.2
[D-Trp^6]LH-RH*	538.6 ± 148.7	1687.5 ± 338.9†	387.8 ± 59†	36 ± 7.4
SB-75, μg/day				
23.8	525.9 ± 114.7	4692.9 ± 531.9‡	909.4 ± 191.9‡	17 ± 1.1
47.6	518.6 ± 154.1	2551.1 ± 560.7†	523.0 ± 127.4†	44 ± 17.6
71.4	578.3 ± 130.5	1515.2 ± 383.3†	291.9 ± 56.3†	50 ± 10.5‡

Results are mean ± SEM; there were six or seven rats per group.
*[D-Trp^6]LH-RH microcapsules were formulated to release 25 μg/day for 30 days.
†$P < 0.01$ vs. control.
‡$P < 0.05$ vs. control.

The effects of treatments on body weight, final tumor weight, and the weight of different organs sensitive to hormonal manipulations are shown in Table 2. The weight of tumors was decreased in a dose-dependent manner by SB-75 treatment, the effect of the highest dose of SB-75 being comparable with that of [D-Trp^6]LH-RH. [D-Trp^6]LH-RH and all three doses of SB-75 significantly decreased the weights of testes, although the effect is less pronounced with 23.8 μg of SB-75 per day. The same pattern was seen in the inhibition of ventral prostate and seminal vesicles weights, the results being highly significant ($P < 0.01$) for all of the treated groups.

The effects of treatment on serum hormone levels are shown in Table 3. LH levels were depressed in all of the treated groups as compared to controls. Serum testosterone was depressed in the group treated with 23.8 μg of SB-75 per day and not detectable in groups that received higher doses of SB-75 or [D-Trp^6]LH-RH. The results of testosterone suppression are reflected in the weights of accessory sex organs, some reduction in weights being achieved with the lowest dose of SB-75, but the maximum inhibition was obtained with the higher doses of SB-75.

FSH levels were uniformly lowered in all of the treated groups and even the lowest dose of SB-75 was as effective as [D-Trp^6]LH-RH. Prolactin levels were lowered by [D-Trp^6]-LH-RH and the highest dose of SB-75, but the lowest dose caused an unexpected increase in prolactin levels. Growth hormone levels were not significantly changed, but the trend in all treated groups was toward lower levels. Insulin-like growth factor I levels, however, were lowered by [D-Trp^6]-LH-RH and SB-75, although the reduction with the highest dose of SB-75 was not significant.

The effects of a prototype of microgranules of SB-75 pamoate were compared with [D-Trp^6]LH-RH in experiment 2. The results on tumor volume are shown in Fig. 2. The tumors in the control group grew during the period of 8 weeks from 5325 ± 715 mm^3 to an average volume of 19,330 ± 4428 mm^3, showing 396% ± 42% increase in the tumor volume and a tumor doubling time of approximately 30 days. Tumor growth was inhibited in both treated groups, but the effect was more pronounced with SB-75 than with [D-Trp^6]LH-RH, especially during the first few weeks of treatment. Tumors in the [D-Trp^6]LH-RH group, from the initial average volume of 5405.8 ± 723.8 mm^3, reached a volume of 11,390 ± 1562 mm^3 (203% ± 26% increase in volume) at the end of the experiment and had a tumor doubling time of 55 days. The tumors in the SB-75 group grew from the initial volume of 5622 ± 1193 mm^3 to the average volume of 10,810 ± 1228 mm^3, corresponding to 194% ± 23% increase in tumor volume and tumor doubling time of 58 days. Tumors in the control group weighed 18.6 g, whereas the tumors of animals treated with [D-Trp^6]LH-RH and SB-75 weighed 10.8 g and 10.2 g, respectively. All of the hormone-dependent organs (testicles, seminal vesicles, and ventral prostates) were greatly decreased in weight compared to controls. The microcapsules of [D-Trp^6]LH-RH and microgranules of SB-75 were very effective in inhibiting serum testosterone, the levels being below the limit of detectability in all animals of both groups. The depression of LH levels from the value of the control group (0.67 ± 0.07 ng/ml) was greater for SB-75 (0.18 ± 0.01 ng/ml) than for the [D-Trp^6]-LH-RH group (0.28 ± 0.04). It is apparent that this formulation of SB-75 was also effective in inhibiting tumor growth and can be favorably compared with [D-Trp^6]LH-RH.

All of the results, taken together, clearly demonstrate that sustained delivery systems of SB-75 depress serum LH and testosterone, decrease weights of hormone-sensitive organs, and inhibit growth of Dunning prostatic carcinoma. No side effects such as edema or anaphylactoid reaction were observed for any of the formulations of SB-75.

DISCUSSION

The present results show the efficacy of the modern LH-RH antagonist SB-75 in inhibiting the growth of transplantable Dunning R3327H carcinoma. Dunning prostate carcinoma is a good model of prostate adenocarcinoma, being histologi-

Table 2. Effect of treatment with microcapsules of agonist [D-Trp^6]LH-RH or antagonist SB-75 on body, organ, and tumor weights in rats bearing Dunning R-3327 prostate cancers

Group	Body weight, g	Testes, g	Ventral prostate, mg	Seminal vesicles, mg	Tumor, g
Control	344.22 ± 11.6	2.48 ± 0.27	298 ± 18.6	427 ± 50.5	6.075 ± 0.89
[D-Trp^6]LH-RH	324.03 ± 10.2	0.53 ± 0.02*	59 ± 8.5*	67 ± 5.6*	1.27 ± 0.23*
SB-75, μg/day					
23.8	319.28 ± 14	1.85 ± 0.26†	99 ± 17.7*	114 ± 26.3*	4.019 ± 0.50†
47.6	307.94 ± 18.6	0.52 ± 0.03*	66 ± 7*	57 ± 3.1*	2.304 ± 0.57*
71.4	322.52 ± 9.9	0.53 ± 0.02*	50 ± 5.8*	58 ± 1.1*	1.505 ± 0.45*

Results are mean ± SEM. The treatment was started 19 weeks after tumor implantation and continued for 8 weeks.
*$P < 0.01$ vs. control.
†$P < 0.05$ vs. control.

Table 3. Serum hormone levels in rats with Dunning R-3327 prostate cancer after 8 weeks of treatment with microcapsules of agonist [D-Trp^6]LH-RH or antagonist SB-75

Group	Testosterone, ng/ml	LH, ng/ml	FSH, ng/ml	Prolactin, ng/ml	GH, ng/ml	IGF-I, pmol/ml
Control	1.76 ± 0.27	0.70 ± 0.10	9.14 ± 0.67	16.97 ± 3.21	9.86 ± 4.73	320.08 ± 5.7
[D-Trp^6]LH-RH	ND	0.29 ± 0.01*	2.61 ± 0.34*	6.33 ± 0.97	7.31 ± 1.67	285.50 ± 8.2†
SB-75, μg/day						
23.8	0.21 ± 0.12*	0.35 ± 0.03*	2.23 ± 0.18*	36.76 ± 6.26*	5.17 ± 0.23	285.87 ± 14.2†
47.6	ND	0.37 ± 0.02*	2.53 ± 0.20*	21.36 ± 3.76	5.09 ± 0.31	259.44 ± 13.5*
71.4	ND	0.37 ± 0.02*	2.33 ± 0.21*	8.88 ± 1.26	8.67 ± 2.80	294.66 ± 5.1

Results are mean ± SEM. ND, nondetectable. GH, growth hormone; IGF-I, insulin-like growth factor I; FSH, follicle-stimulating hormone.
*$P < 0.01$ vs. control.
†$P < 0.05$ vs. control.

cally and biochemically similar to human tumors. Its hormonal dependency makes it also suitable for testing hormonal manipulations, including the use of LH-RH analogs (1, 7, 13, 16–18, 22, 23). We have previously shown that chronic administration of agonist [D-Trp^6]LH-RH or older antagonists of LH-RH to Copenhagen rats bearing the Dunning R-3327 carcinoma inhibited tumor growth and decreased the levels of testosterone, LH, FSH, the weight of prostate, seminal vesicles, and testicles (1, 7, 13, 16–18, 22, 23). The present study, using sustained-release microcapsules and microgranules of the modern antagonist SB-75, extends these findings. The sustained-delivery systems are more convenient and more efficacious for delivering therapeutic doses of agonists and antagonists than multiple daily administration (12). Clinical use of sustained-release formulation of agonists of LH-RH was previously shown to be efficacious (19). The suppression of gonadal functions achieved by administration of LH-RH agonists and antagonists is equivalent to chemical castration and provides an efficient approach for the treatment of prostate cancer and other sex hormone-dependent tumors (1, 3, 16, 20, 21). The comparison of the efficacy of the antagonist SB-75 and the agonist [D-Trp^6]LH-RH in rats with prostate cancer shows that the antagonist might be a better alternative, because of immediate inhibition of the pituitary-gonadal axis, which clinically would avoid the flare-up of disease. The effectiveness of SB-75 was also demonstrated in estrogen-sensitive MXT mammary adenocarcinoma in BDF mice, the inhibition of tumor growth induced by SB-75 being superior to that caused by [D-Trp^6]LH-RH or Tamoxifen or their combination (20). In breast cancer, as in prostate cancer, the advantage of antagonists, in addition to a possible better inhibition of tumor growth, could be also based on the fact that they inhibit LH, FSH, and sex steroid secretions

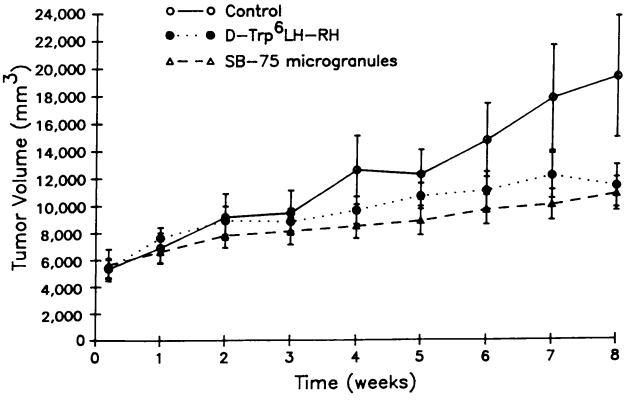

FIG. 2. Effect of treatment with microcapsules of [D-Trp^6]LH-RH or microgranules of antagonist SB-75 on tumor growth in rats with Dunning R3327 prostate cancer. The microcapsules of [D-Trp^6]LH-RH liberated 25 μg/day, whereas the prototype microgranules of SB-75 were estimated to release 45–60 μg/day. Vertical lines indicate the SEM.

from the start of the administration. The use of antagonists in the treatment of cancer would avoid the transient stimulation of the release of gonadotropins and sex steroids that occurs initially in response to LH-RH agonists (1, 3, 10, 11, 20). The antagonist SB-75 was also demonstrated to inhibit tumor growth and promote programed cell death (apoptosis) in *N*-nitrosobis(2-oxopropyl)amine-induced pancreatic cancers in hamsters (27).

The inhibition of LH, FSH, and testosterone levels in men by antagonistic analog SB-75 was recently shown by our group.‡ Preliminary results during its administration in patients with advanced prostatic carcinoma show improvement of clinical signs and symptoms with no accompanying side effects.‡ Direct effects of modern antagonistic analogs, such as SB-75, on tumors should be explored, since the existence of receptors for LH-RH was demonstrated in Dunning prostate tumors and other tumors in animals (1, 3, 28, 29) and in human prostate cancer (29, 30). These receptors were characterized and their possible role in mediating direct effects of LH-RH analogs on tumors was suggested (29, 30). Direct inhibition of growth of estrogen-independent MDA-MB-231 human mammary tumor cell line by antagonists of LH-RH was demonstrated *in vitro* (31). The inhibitory effects of agonist [D-Trp^6]LH-RH and the LH-RH antagonists, including SB-75, on [^{3}H]thymidine incorporation were similarly demonstrated on prostatic cancer cell line LNCaP in culture.§

Since the somatostatin analog RC-160 was also shown to inhibit [^{3}H]thymidine incorporation into LNCaP cells§ and its effects were shown to be synergistic with [D-Trp^6]LH-RH in suppressing the growth of Dunning prostate cancers in rats (16), the combination treatment of LH-RH antagonist SB-75 with RC-160 should be also explored in prostate cancer models.

The combination of chemotherapy and hormonal manipulation with LH-RH agonists or antagonists (1, 17, 18) or castration (2) is feasible and encouraging in producing a potential cure in hormone-sensitive cancers (2). The efficacy of combined treatment is greater than that of either agent alone (17, 18) and can be increased by proper timing early in the evolution of cancer (32). Another approach to a potential cure could consist of use of LH-RH analogs carrying cytotoxic radicals (33, 34), which could be targeted to prostate and other cancers that have membrane receptors for LH-RH.

In conclusion, our results may provide a stimulus for further studies with LH-RH antagonists in prostate cancer. Although the microcapsules and microgranules of SB-75

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§Gattani, A., Brower, S., Platica, M., Schally, A. V. & Hollander, V., Proceedings of Annual Meeting of American Association of Cancer Research, May 23–26, 1990, Washington, abstr. 1299, p. 219.

reported herein represented only prototype batches, our work indicates that the improved sustained delivery formulations of the LH-RH antagonist SB-75 should be capable of maintaining a therapeutic level of the peptide for several weeks. Further work can lead to clinical batches of SB-75 microcapsules or microgranules suitable for the therapy of hormone-sensitive tumors and endocrine and gynecologic disorders.

We dedicate this manuscript to the memory of Marion Stevens of Papanicolaou Cancer Research Institute, Miami, recently deceased. Over a period of years, her expert help and kind cooperation in supplying experimental animals bearing prostate cancers made possible the successful completion of many studies, including this one. Many cancer researchers are indebted to Marion Stevens for her contributions. A generous gift of SB-75 microgranules, a result of collaboration between Asta Pharma, Frankfurt/Main (Germany), and Cytotech S.A., Martigny (Switzerland), is greatly appreciated. We are grateful to Mr. Weldon Carter for his assistance in the preparation of the manuscript. We thank the National Hormone and Pituitary Program (NHPP), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health for the gifts of materials used in radioimmunoassay. The work described in this paper was supported by National Institutes of Health Grant CA 40003 and by the Medical Research Service of the Veterans Affairs (to A.V.S.) and the G. Harold and Leila Mathers Charitable Foundation.

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