Sustained Release Formulations of Luteinizing Hormone-releasing Hormone Antagonist SB-75 Inhibit Proliferation and Enhance Apoptotic Cell Death of Human Prostate Carcinoma (PC-82) in Male Nude Mice

Tommie W. Redding, Andrew V. Schally, Sinisa Radulovic, Slobodan Milovanovic, Karoly Szepeshazi, and John T. Isaacs

Endocrine, Peptide and Cancer Institute, Veterans Affairs Medical Center, and Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana 70116 [J. T. I., A. V. S.], and Department of Urology, James Buchanan Brady Urological Institute, The Johns Hopkins Hospital, Baltimore, Maryland 21205 [J. T. I.]

ABSTRACT

The function of the pituitary-gonadal axis in normal (immunocompetent) and nude (immunocompromised) mice, like that of other species, can be suppressed by luteinizing hormone-releasing hormone (LH-RH) agonists and antagonists administered by continuous release systems and, therefore, nude mice provide a valuable model for investigation of the effects of LH-RH analogues on growth of xenografts of human cancers. To extend our findings further, we treated male nude mice bearing xenografts of human prostate adenocarcinoma PC-82, for 42 days, with sustained release formulations (microcapsules or microgranules) of the agonist [d-Trp6]-LH-RH, the antagonist [Ac-d-Nal(2), d-Phe(4)C]LH-RH, or the somatostatin analogue t-Phe-Cys-Tyr-d-Trp-Lys-Val-Cys-Trp-NH2 (RC-160). At necropsy, in mice given microcapsules releasing 50 ìg/day of [d-Trp6]-LH-RH, tumor weight and volume were significantly decreased, compared with control mice, and weights of testes, ventral prostate, and seminal vesicles were also reduced in this group. In mice which received microgranules liberating 50 ìg/day of antagonist SB-75, there was a greater decrease in tumor weight and volume than that produced by the agonist and a significant reduction in the weight of the testes and accessory sex organs. Histological parameters also demonstrated significant tumor inhibition, with the best results being obtained by treatment with the antagonist SB-75. The tumor inhibition induced by SB-75 was demonstrated to be due to decreased cellular proliferation, with enhanced cellular death (i.e., apoptosis) of the PC-82 cells. Microcapsules releasing 50 ìg/day of RC-160 decreased tumor weight and volume by 23% and 28%, respectively, but this reduction was not significant. Serum levels of testosterone were decreased by 90% in mice given the LH-RH agonist and by 94% in response to the antagonist SB-75. Serum levels of prostate-specific antigen were significantly lower in mice treated with LH-RH analogues, with the antagonist SB-75 causing a greater reduction. A ratio of prostate-specific antigen to tumor weight suggests that levels of serum prostate-specific antigen may be correlated with tumor mass. Using sensitive multipoint micromethods, one class of binding sites for LH-RH, with a dissociation constant of 7.8 ± 1.2 nM and a maximal binding capacity of 126.4 ± 23.1 fmol/mg protein, was found in the control tumors. Tumors from mice treated with either LH-RH agonist or antagonist, but not somatostatin analogue RC-160, showed a significant reduction in maximal binding capacity for LH-RH, compared to control tumors. These data clearly demonstrate, for the first time, that the growth of human prostate cancers in nude mice can be inhibited by administration of modern antagonists of LH-RH, like SB-75.

Received 10/18/91; accepted 2/26/92.

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1 The work was supported by NIH Grant CA 40003 and by the Medical Research Service of the Veterans Affairs (to A. V. S.). The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

2 To whom requests for reprints should be addressed, at VA Medical Center, 1601 Perdido Street, New Orleans, LA 70146.

INTRODUCTION

Most experimental studies on prostate cancer involving LH-RH agonists (1-5) and, more recently, LH-RH antagonists (6) were carried out using the hormone-dependent Dunning R3327H prostate tumor transplanted into Copenhagen F-1 rats. This model has enhanced our knowledge about the effects of LH-RH analogues on tumor biology and contributed to the development of new approaches to hormonal therapy (7, 8). However, studies using rats are limited to transplantable or carcinogen-induced rodent tumors and suffer the disadvantage of being of non-human origin. Human tumors transplanted into athymic nude mice may serve as a more appropriate in vivo model for studying tumor growth, since they maintain many of their cellular characteristics, such as karyotype, morphological and histological appearances, and the production of specific enzymes, antigens, and hormones (9). However, it had been incorrectly stated that the pituitary-gonadal axis of conventional and nude mice, unlike that of other species, cannot be suppressed by the administration of LH-RH agonists and antagonists (10-13). This view led many investigators to abandon the use of mice for testing the effect of new LH-RH analogues on the inhibition of growth of sex hormone-dependent cancers.

Initial attempts by our group to demonstrate that normal female mice are sensitive to the inhibitory effect of LH-RH agonists involved long term treatment with delayed delivery systems of [d-Trp6]-LH-RH. After 5 months of treatment with small doses of this analogue, complete suppression of the pituitary-gonadal axis was achieved in female mice (14). Subsequently, we expanded our studies to long term treatment of male nude mice with sustained release formulations of the agonist [d-Trp6]-LH-RH and two new potent antagonists, SB-30 and SB-75, administered by osmotic minipumps, which permitted a continuous release of the peptides into the circulation for 30 days (15). Major, highly significant decreases in weights of the testes, ventral prostate, and seminal vesicles and reduction in levels of serum LH and testosterone in groups treated with antagonists SB-30, SB-75, or [d-Trp6]-LH-RH, compared to control mice, clearly documented that the pituitary-gonadal axis of nude mice can be inhibited by LH-RH agonists and antagonists if they are administered by continuous release systems (15).

The PC-82 human prostate adenocarcinoma tumor line transplanted into male nude mice maintains many properties comparable to those seen in a clinical setting (13, 16-19). This tumor line shows a slow growth rate, expresses nuclear andro-
gen receptors, and is hormone dependent. PC-82 also secretes prostatic acid phosphatase and is antigenic for prostate-specific antigen (16, 17, 19). Previous studies demonstrated that androgen ablation, induced by surgical castration, resulted in the regression of PC-82 tumors in nude mice and that this regression was due to both a decrease in proliferation and enhanced death of PC-82 cells (18). In addition, the androgen ablation-induced death of the cancer cells was demonstrated to occur via the energy-dependent process of programmed cell death (i.e., apoptosis). Histologically, apoptosis is defined by morphological changes which characteristically involve chromatid condensation, cell surface blebbing, and eventually cellular fragmentation into a cluster of membrane-bound apoptotic bodies. We decided to carry out long term experiments in nude male mice bearing xenografts of human prostate cancer PC-82, in order to establish the utility of the antagonist SB-75 and the somatostatin analogue RC-160 for the treatment of prostate cancer. We evaluated microgranules of the antagonist SB-75 and microcapsules of the agonist [d-Trp6]-LH-RH and the somatostatin analogue RC-160, to determine whether such sustained release formulations could effectively inhibit the proliferation of the PC-82 prostate cancer cells and induce their apoptotic death.

MATERIALS AND METHODS

Athymic nude (nu/nu) male mice, approximately 6 weeks of age upon arrival, were obtained from the National Cancer Institute (Bethesda. MD). They were housed in sterile plastic cages and placed in a continuous laminar air flow hood, under an automatic light/dark schedule (12/12 h). Commercially available pelleted diet, bedding, water bottles, and water were sterilized. All manipulations of mice were carried out, with sterile-gloved hands, under a horizontal laminar air flow hood (15). Ten to 12 animals were used for each experimental group.

The PC-82 human prostate line was originally developed by Hoehn et al. (16) in the Department of Urology, Erasmus University, (Rotterdam, The Netherlands). This human prostatic tumors line has been described as being a slow growing, moderately differentiated, hormone-dependent adenocarcinoma, which secretes immunoreactive prostatic acid phosphatase and prostatic-specific antigen (13, 16–19). When donor tumors from mice transplanted with the PC-82 tumor had reached a tumor volume of about 100 mm3, the mice were sacrificed and the tumors were carefully excised under sterile conditions, minced with scissors, and then gently passed through two sterile wire mesh gauze strainers (1.5 and 0.5 mm). This slurry of dispersed tissue was centrifuged at 100 x g for 5 min. The wash procedure was repeated twice, and the resulting cell pellet was diluted with an equal volume of Matrigel (Collaborative Research, Bedford, MA) (20).

Two hundred µl of this cell suspension, representing about 20 mg wet weight, were injected s.c., with the animals under light anesthesia (metofane; Pittman-Moore, Washington Crossing, NJ), into the anterior-dorsal region, using an 18-gauge needle. This procedure resulted in a 97% “take” of tumor. After about 35 to 40 days, small tumors were palpable. Tumors were measured weekly with microcalipers, as previously described (1–5). After 60 days, the tumors had a mean volume of about 128 mm3. The mice were then randomized and divided into four groups of 10 to 12 animals each.

Sterile microcapsules of the agonist [d-Trp6]-LH-RH (3–5, 14), microgranules of the antagonist SB-75 (Ac-D-Nal(2),D-Phe(4)NH2,D-Pal(3),D-Trp(6)-LH-RH) (6, 21, 23), and microcapsules of the somatostatin analogue RC-160 ([D-Phe-Cys-Tyr-D-Val-Cys-Trp-NH2]5) consisted of the peptides distributed within a polymeric matrix of biodegradable poly(ε-lactide-co-glycolide), as previously reported (3–6, 14, 21). Microcapsule formulation of the LH-RH agonist [d-Trp6]-LH-RH was prepared by Dr. P. Orsolini at Cytotech (Martigny, Switzerland). Microgranules of SB-75 were also prepared by Dr. P. Orsolini. Microcapsules of RC-160 (lot RCR-90–22), similarly, were made at Cytotech. Using sterile techniques, aliquots of microcapsules or microgranules were weighed, in doses calculated to release a specific amount of the analogues/day, and were transferred to sterile disposable syringes (3, 5).

These microcapsule or microgranule preparations were suspended in 0.5 ml sterile injection vehicle, containing 2% (w/v) carboxymethylcellulose 7LF (Hercules Inc, Wilmington, DE) and 1% (v/v) Tween 80 (polyoxyethylene polysorbate monolaurate) (Matheson, Coleman and Bell) in water. This suspension was mixed and injected s.c. through an 18-gauge needle. [d-Trp6]-LH-RH microcapsules were injected once per month, SB-75 microgranules every 3 weeks, and RC-160 microcapsules every 2 weeks. Mice were monitored carefully for infection or any unusual reaction to the drugs during the course of the experiment. Those mice showing signs of infection or other complications were removed from the experiment. Tumor volumes were measured weekly. On day 42, mice were anesthetized with metofane and decapitated. Trunk blood was collected, and serum was separated for further analyses. Body weights were recorded, and various organs were removed, cleaned, and weighed along with the tumors. Pieces of tumor tissue were fixed in neutral buffered formalin for histological examination. Specimens were embedded in Paraplast (Oxford Labware, St. Louis, MO), and 6-µm-thick sections were cut and stained with hematoxylin and eosin. The numbers of mitotic and apoptotic cells (18) per 1000 tumor cells were determined (mitotic and apoptotic indices, respectively). For the determination of connective tissue content of tumors, a microscope-ocular net was used, and the crossing points of this net that hid or coincided with connective tissue in sections were counted. The ratio of these points to the number of all points represented the ratio of the volume of connective tissue to the volume of tumor tissue. For demonstration of the nucleolar organizer region in tumor cell nuclei, the AgNOR method of Chiu et al. (22) was used. The silver-stained black dots in 50 cells of each tumor were counted, and the AgNOR number per cell was calculated. All histological counts were carried out without knowledge of treatment. For immunohistochemical detection of prostate acid phosphatase in tumor slides, a Milab immunocytochemistry kit was used (Milab, Malmö, Sweden).

Serum levels of testosterone were measured by a specific radioimmunoassay, as previously described (23). The interassay and intraassay coefficients of variation were 10.8% and 7%, respectively. The concentration of prostate-specific antigen in serum (19) was measured using a radioimmunoassay kit (Hybritech, San Diego, CA).

LH-RH, somatostatin, IGF-1, and EGFR receptors in the PC-82 prostate tumors were measured as previously described (24–26). The LIGAND PC computerized curve-fitting program of Munson and Rodbard (27) was used to determine the types of receptors binding, Kd values, and the Bmax of receptors. Other statistical analyses of the tumor data were performed using Duncan’s new multiple range test (2–5).

RESULTS

Sixty days after transplantation, the RC-82 tumors had grown to a volume of about 128 mm3, and the animals were then randomized and divided into four groups of 10 mice each. The control mice received only the vehicle. The second group was given s.c. injections of microcapsules designed to release [d-Trp6]-LH-RH, at a rate of about 25 µg/day, for 30 days. The third group were given s.c. injections of microgranules of antagonist SB-75, which released the peptide at about 50 µg/day over a 3-week period. A fourth group of mice were treated with s.c. injections of microcapsules designed to release 50 µg/day of somatostatin analogue RC-160 for approximately 2 weeks. Treatment was continued for 42 days.

Fig. 1 shows the tumor volume, as measured, at weekly intervals in the course of the experiment. Tumor volume in mice treated with microcapsules of [d-Trp6]-LH-RH was sig-
significantly decreased by 65% at day 35 and by 67% by day 42, compared to control animals. Treatment with SB-75 microcapsules significantly inhibited tumor growth by 55% on day 28 and 78% by day 42. There were no significant changes in tumor volume, compared to controls, during the administration of microcapsules of somatostatin analogue RC-160, although the final volume was smaller than in controls. Tumor doubling time, calculated from growth curves, was 17 ± 1.4 days in controls. In mice treated with microcapsules of [D-Trp6]-LH-RH or somatostatin analogue RC-160, doubling time was extended to 35 ± 5.7 days and 23 ± 4.5 days, respectively. Tumor doubling time in mice receiving SB-75 microgranules could not be estimated, since after 42 days of treatment the tumors had regressed by ~25% of their starting size at day 0.

Fig. 2 illustrates tumor growth expressed as percentage change in tumor volume, measured at weekly intervals, after the data were normalized for variation among initial tumor volumes on day 0. Tumor growth in all three experimental groups, as measured by percentage change from the initial tumor volume, at day 0, was inhibited within 7 days, compared to controls. However, only nude mice treated with antagonist SB-75 demonstrated an involution of tumor mass and a negative percentage change (i.e., 25% regression) during the course of treatment. This group of mice also showed the greatest decreases in tumor volume and weight (Table 1). The administration of somatostatin analogue RC-160 or the agonist [D-Trp6]-LH-RH also slowed the rate of growth, but only the latter analogue caused a significant reduction in tumor weight and volume at the conclusion of the experiment (Fig. 2, Table 1).

Table 1 records body and organ weights, as well as tumor weights, at necropsy, tumor burden, and final tumor volume after 42 days of treatment with microcapsules of RC-160, microcapsules of [D-Trp6]-LH-RH, or microparticles of SB-75. Tumor weights were decreased by 80% in mice receiving antagonist SB-75 for 42 days, compared to control mice (P < 0.01), and there was also a significant reduction in the weights of the testes, ventral prostate, and seminal vesicles in this group. Tumor volume and tumor burden were significantly diminished in mice receiving SB-75 microgranules, compared to mice receiving vehicle alone. Treatment of mice bearing the PC-82 tumor with [D-Trp6]-LH-RH microcapsules resulted in a 63% reduction in tumor weight, compared to control mice (P < 0.05). Tumor volume and tumor burden were decreased by 67% (P < 0.05) and 62% (P < 0.01), respectively, in this group of mice, and testes and accessory sex organs were significantly diminished. In mice receiving microcapsules of somatostatin analogue RC-160, final tumor volume and tumor weight were reduced, but the difference was not significant, compared to controls.

Histologically, the tumors were adenocarcinomas, presenting a cribriform or papillary pattern in some areas. The glands were lined by columnar epithelial cells that were arranged in two or three layers. The cells had large, pale, oval nuclei containing some small nucleoli. The glands were supported by a scant fibrous stroma that comprised about 13% of the volume of the tumor tissue in control animals (Fig. 3). The amount of stroma was increased in all treated tumors, with the greatest significant increase in those treated with SB-75 (Fig. 4). In these tumors, the stroma was more cellular, containing fibroblasts, histiocytes, myoepithelial cells, and inflammatory cells. In the treated tumors, the glands were smaller and their epithelial layer was thinner, consisting of only one layer of cuboidal cells in some glands. Proliferation of the PC-82 cells, as monitored by number of mitoses, was decreased significantly in the tumors treated with agonist [D-Trp6]-LH-RH or somatostatin analogue RC-75, with the greatest reduction occurring after treatment with the antagonist. The number of AgNORs in tumor cell nuclei decreased significantly in the groups receiving SB-75 or [D-Trp6]-LH-RH but was only slightly smaller in the tumors treated with RC-160. Cells undergoing apoptosis were found in all tumors, even in the controls. However, a significant enhancement of apoptosis was observed in all treated groups, with the highest apoptotic index values appearing in tumors treated with SB-75, being about 400% of control values (Fig. 5). Histological data are summarized in Table 2. Prostatic acid phosphatase was detected in the cytoplasm of tumor cells, as well as in secreted material in tumorous glands, in both control (Fig. 6A) and treated tumors (Fig. 6B).

The levels of serum testosterone in control mice and in mice treated with the agonist [D-Trp6]-LH-RH, antagonist SB-75, or somatostatin analogue RC-160 are shown in Table 3. Serum testosterone levels in mice treated with SB-75 microgranules decreased by 94% (P < 0.01), while [D-Trp6]-LH-RH microcapsules caused 90% (P < 0.05) reduction in testosterone, compared to control mice. There was a statistically significant fall in PSA levels in serum of mice treated with SB-75 microparticles or [D-Trp6]-LH-RH microcapsules (97% and 84%, respectively), compared to control mice (Table 3). RC-160 microcapsules reduced PSA levels by 20%, which was not statistically significant. When PSA serum levels were adjusted by tumor weight, it appeared that PSA concentrations may be directly related to tumor mass.
The characteristics of LH-RH receptor binding in the PC-82 tumor tissue were analyzed following treatment with these analogues, and the results are presented in Table 4. [D-Trp⁶]-LH-RH showed binding to one class of high affinity and low capacity binding sites (Kd = 7.8 ± 1.2 x 10⁻⁹ M; Bmax = 126 ± 23.1 fmol/mg of membrane protein) in tumor membranes from untreated control mice. Therapy with microcapsules of [D-Trp⁶]-LH-RH or microgranules of antagonist SB-75 significantly reduced Bmax in tumor membranes, indicating a down-regulation of the LH-RH receptors. Chronic administration of somatostatin analogue did not change the characteristics of [D-Trp⁶]-LH-RH binding.

**DISCUSSION**

The concept that malignant prostate tumors are primarily an overgrowth of adult epithelial cells, which could be inhibited by the elimination of androgens through castration or administration of estrogens, was first described by Huggins and Hodges in the 1940s (28). Androgen deprivation still represents the standard treatment for prostatic cancer (29). A new and radically different endocrine therapy for prostate cancer and other sex hormone-dependent or -sensitive cancers is based on superactive agonists of LH-RH (30, 31). Chronic administration of these analogues causes a marked inhibition of pituitary and gonadal function, a down-regulation of pituitary LH-RH receptors, and a marked decline in sex hormone levels (30-33). The potential utility of LH-RH agonists in the treatment of prostate carcinoma was first demonstrated in studies in rat models of prostate cancer (1, 30). The finding that prolonged treatment with agonistic analogues of LH-RH can result in chemical castration prompted us to use this method in an attempt to induce the regression of prostate tumors in rat models (1). [D-Trp⁶]-LH-RH treatment of male rats bearing the Dunning R3327H prostate adenocarcinoma reduced tumor volume and decreased tumor weight, compared to untreated controls, and lowered serum LH, follicle-stimulating hormone, and testosterone levels (1).

The demonstration that [D-Trp⁶]-LH-RH inhibits the growth of prostate tumors in rats led to successful clinical trials (35-40). However, the duration of remission in patients with prostate cancer is limited, because various modalities of androgen deprivation, including those based on LH-RH agonists (4, 5, 31, 37, 38, 41), do not prevent the ultimate growth of hormone-independent cells (8). Combination therapy or use of other types of analogues might forestall this phenomenon and prolong survival (4, 5, 31). The development of LH-RH antagonists, somatostatin analogues, and cytotoxic LH-RH analogues is actively proceeding (21, 31, 41, 42). 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 (6, 23, 25, 31, 42). To determine the efficacy of new analogues, they must be tested singly and in combination, not only in rats with Dunning prostate tumors but also in other prostate cancer models, especially in nude mice bearing transplanted human cancers (9).

However, several reports incorrectly claimed that the function of the pituitary-gonadal axis in normal (immunocompetent) and nude (immunocompromised) mice, unlike that of other species, cannot be suppressed by the administration of LH-RH agonists or antagonists (10-13). This view led many investiga-
tors to abandon the use of mice for testing the effects of new LH-RH analogues on the inhibition of growth of sex hormone-dependent cancers. Many factors may have contributed to the inability of other investigators to suppress the pituitary axis in both normal and athymic mice treated with analogues of LH-RH (10–13). The short half-lives of these peptides, along with the high metabolic rate in mice, may not allow a long term occupancy of pituitary LH-RH receptors when these analogues are administered once per day or even several times per day (14, 15). Data in mice show that <40% LH-RH receptor occupancy is adequate for maximal hormone secretion in vivo (43, 44). The probability of prolonged occupancy of pituitary LH-RH receptors is greater when analogues are administered in a sustained delivery formulation, which allows continuous release into the circulation, thereby maintaining a higher level of the peptide at the receptor site. Although agonists and antagonists of LH-RH act by different mechanisms (30–33), a prolonged effect on LH-RH receptors, by both agonists and antagonists, would probably also be required in mice to obtain pituitary and gonadal inhibition. Initial attempts by our group to demonstrate that normal female mice are sensitive to the inhibitory effect of LH-RH agonists involved long term treatment with delayed delivery systems of [α-Trp^6]-LH-RH (14). After 5 months of treatment with small doses of this agonist, a suppression of the pituitary-gonadal axis was demonstrated (14). Subsequent studies using male nude mice treated with sustained release formulations of the agonist [α-Trp^6]-LH-RH or the antagonists SB-30 and SB-75 showed a complete inhibition of the pituitary-gonadal axis, characterized by a decrease in weights of the testes and accessory sex organs and a dramatic reduction in levels of serum LH and testosterone (15).

Previously, van Steenbrugge et al. (13) treated male nude mice bearing the PC-82 human adenocarcinoma with the LH-RH antagonist ORG-30276, at a dose of 100 μg/day for 21 days, s.c., but the short treatment period with once per day injections of this old antagonist failed to alter significantly tumor doubling time, tumor weight, or the weight of the testes and accessory sex organs. Since an inhibition of the pituitary-gonadal axis was not overtly demonstrated, van Steenbrugge et al. (13) concluded that nude mice were not a suitable model for studying the effects of LH-RH antagonists on this androgen-dependent prostate tumor. In the present study, in nude mice bearing this same transplantable human prostate tumor (PC-82), treatment with agonist [α-Trp^6]-LH-RH or antagonist SB-75, administered as sustained release formulations which pro-

| Table 2 Effect of treatment with RC-160, SB-75, and [α-Trp^6]-LH-RH on histological parameters of RC-82 tumors xenografted to nude mice |
|-----------------|-----------------|-----------------|-----------------|
| Treatment group | Volume of stroma in tumors (%) | No. of mitoses/1000 cells | No. of apoptotic cells/1000 cells | No. of AgNORs/tumor cell |
| Control         | 12.9 ± 2.0^*     | 3.00 ± 0.44      | 6.00 ± 1.18      | 10.37 ± 0.30          |
| RC-160          | 15.5 ± 2.1       | 2.50 ± 0.43      | 12.50 ± 2.41^*   | 9.98 ± 0.37           |
| SB-75           | 40.4 ± 2.9^*     | 0.75 ± 0.16^*    | 24.50 ± 2.57^*   | 8.96 ± 0.16^*         |
| [α-Trp^6]-LH-RH | 19.7 ± 2.7       | 1.14 ± 0.26^*    | 13.86 ± 1.42^*   | 9.54 ± 0.33^*         |

^* Mean ± SE.
^ P < 0.05.
^* P < 0.01.
^** P < 0.05, with Student's t test.

Fig. 5. Several apoptotic cells (arrows) in a PC-82 tumor after treatment with SB-75. H & E. × 300.

Fig. 6. Prostatic acid phosphatase in the cytoplasm of tumorous epithelial cells, detected by immunohistochemistry in PC-82 prostate cancers from a control mouse (A) and from a mouse treated with SB-75 (B). No counterstain. × 300.
prostate cancer xenografts after in vivo treatment of nude mice with microcapsules. Values shown are mean ± SE.

Over an extended period of time, significantly inhibited tumor growth of human prostatic cancer, murine and human breast cancer (45), and other sex hormone-dependent cancers. The results presented here clearly demonstrate that administration of sustained release formulations of LH-RH antagonist SB-75 or agonist [d-Trp⁶]LH-RH inhibits the growth of the human PC-82 prostate tumor transplanted into male nude mice. This growth inhibition involves both decreased cellular proliferation and enhanced cellular death via apoptosis. Nude mice bearing xenografts of PC-82 and other cancer lines should provide a valuable model for studying the effects of LH-RH agonists and antagonists, and other analogues, on tumor biology.

ACKNOWLEDGMENTS

We thank ASTA-Pharma A.G. (Frankfurt/Main, Germany) for a generous supply of antagonist SB-75 and Debiopharm S.A. (Lausanne, Switzerland) for microcapsules of agonist [d-Trp⁶]LH-RH and somatostatin analogue RC-160. A generous gift of SB-75 microcapsules, a result of collaboration between ASTA-Degussa, (Frankfurt/Main, Germany) and Cytotech S.A. (Martigny, Switzerland), is greatly appreciated. We are grateful to Dr. Kate Groot and Lynette Hinojosa-Forero for radioimmunoassays, Annamaria B. Zsigo for assistance in histology, E. Monje for receptor assays, and Harold Valerio for experimental assistance. It is a pleasure to acknowledge useful discussions and valuable advice from Professor Juergen Engel.

REFERENCES


3. Redding, T. W., Schally, A. V., Tice, T. R., and Meyers, W. E. Long-acting somatostatin analogue RC-160 was used in our study because we have shown previously that microcapsules of RC-160 inhibit the growth of Dunning prostate tumors (5). The finding that tumor volume and weight in the group treated with RC-160 in our present study did not differ significantly from those in control animals may be due to the pharmacokinetics of the preparation of RC-160 microcapsules used in this work (5, 41). Somatostatin analogues such as RC-160 appear to interfere with the secretion or action of growth factors like IGF-I and EGF (5, 41, 42).

The results presented here clearly demonstrate that administration of sustained release formulations of LH-RH antagonist SB-75 or agonist [d-Trp⁶]LH-RH inhibits the growth of the human PC-82 prostate tumor transplanted into male nude mice. This growth inhibition involves both decreased cellular proliferation and enhanced cellular death via apoptosis. Nude mice bearing xenografts of PC-82 and other cancer lines should provide a valuable model for studying the effects of LH-RH agonists and antagonists, and other analogues, on tumor biology.

Table 3. Serum testosterone and prostate-specific antigen levels in nude mice bearing the human prostate tumor PC-82, after treatment with sustained delivery systems of RC-160, SB-75, or [d-Trp⁶]-LH-RH.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum testosterone (ng/ml)</th>
<th>PSA (ng/ml)</th>
<th>PSA/tumor weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.81 ± 1.47*</td>
<td>175 ± 4.0</td>
<td>0.94 ± 0.2</td>
</tr>
<tr>
<td>RC-160 microcapsules</td>
<td>2.14 ± 0.78</td>
<td>142 ± 62</td>
<td>1.37 ± 0.58</td>
</tr>
<tr>
<td>SB-75 microgranules</td>
<td>0.22 ± 0.07*</td>
<td>5 ± 1*</td>
<td>0.05 ± 0.01*</td>
</tr>
<tr>
<td>[d-Trp⁶]-LH-RH microcapsules</td>
<td>0.38 ± 0.13*</td>
<td>28 ± 10*</td>
<td>0.20 ± 0.05*</td>
</tr>
</tbody>
</table>

* Mean ± SE.

Statistically significant differences versus control (P < 0.05).

Table 4. Characteristics of [d-Trp⁶]-LH-RH binding in membranes of PC-82 prostate cancer xenografts after in vivo treatment of nude mice with microcapsules of somatostatin analogue RC-160, microgranules of antagonist SB-75, or microcapsules of [d-Trp⁶]-LH-RH.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Kd (x 10⁻⁷ M)</th>
<th>Bmax (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.8 ± 1.2</td>
<td>126 ± 4</td>
</tr>
<tr>
<td>RC-160 microcapsules</td>
<td>8.6 ± 2.3</td>
<td>149 ± 8</td>
</tr>
<tr>
<td>SB-75 microgranules</td>
<td>1.4 ± 0.2*</td>
<td>12.9 ± 5.8*</td>
</tr>
<tr>
<td>[d-Trp⁶]-LH-RH microcapsules</td>
<td>2.6 ± 0.1*</td>
<td>39.7 ± 11.6*</td>
</tr>
</tbody>
</table>

* Statistically significant differences versus control (P < 0.05).


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