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Inhibition of growth of OV-1063 human epithelial ovarian cancer xenografts in nude mice by treatment with luteinizing hormone-releasing hormone antagonist SB-75

(analogs of luteinizing hormone-releasing hormone/selective medical hypophysectomy/ovarian carcinoma/gynecologic cancers)

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ABSTRACT **Female athymic nude mice bearing xenografts of OV-1063 human epithelial ovarian cancer cell line were treated with potent luteinizing hormone (LH)-releasing hormone (LH-RH) antagonist SB-75 {Cetrorelix; [Ac-D-Nal(2)[1], D-Phe(4 Cl)[2], D-Pal(3)[3], D-Cit[6], D-Ala[10]]LH-RH in which Ac-D-Nal(2) = *N*-acetyl-3-(2-naphthyl)-D-alanine, D-Phe(4Cl) = 4-chloro-D-phenylalanine, D-Pal(3) = 3-(3-pyridyl)-D-alanine, and D-Cit = D-Citrulline} or with the agonist [D-Trp[6]]LH-RH. In the first experiment, SB-75 and [D-Trp[6]]LH-RH were administered in the form of microcapsules releasing 60 and 25 μg/day, respectively. In the second study, the analogs were given by daily s.c. injections in doses of 100 μg/day. In both experiments, tumor growth, as measured by reduction in tumor volume, percentage change in tumor volume, tumor burden, and increase in tumor doubling time, was significantly inhibited by treatment with SB-75 but not with [D-Trp[6]]LH-RH. Uterine and ovarian weights were reduced and serum LH levels decreased by administration of either analog. Chronic treatment with SB-75 greatly reduced the concentration of receptors for epidermal growth factor and insulin-like growth factor I in tumor cell membranes, a phenomenon that might be related to tumor growth inhibition. It is possible that the antitumoral effects of SB-75 on OV-1063 ovarian cancers are exerted not only through the suppression of the pituitary–gonadal axis, but also directly. In view of its strong inhibitory effect on the growth of OV-1063 ovarian cancers *in vivo*, the potent LH-RH antagonist SB-75 might be considered for possible hormonal therapy of advanced epithelial ovarian carcinoma.**

Epithelial ovarian cancer is the most common cause of death from gynecologic malignancies in the Western World (1). It is estimated that in 1992 ≈21,000 new cases of ovarian cancer were diagnosed in the United States, and that ≈13,000 deaths from this disease occurred (2). Treatment of ovarian cancer is based on surgery and chemotherapy, but the long-term survival rate is disappointing (1, 3, 4). New therapeutic approaches to ovarian cancer should be explored to improve the response (1).

The normal ovary is a hormone-dependent organ and receptors for estrogen, progesterone, androgen, luteinizing hormone (LH), and LH-releasing hormone (LH-RH) are found in ovarian cancers (3, 5–7). It was reported that gonadotropins and estradiol increased the growth rate of some ovarian cancer cell lines (8, 9), but the exact mechanism of endocrine regulation of the growth of ovarian cancer is still unclear. Some experimental and clinical findings indicate that the suppression of the secretion of gonadotropins produced by LH-RH agonists may inhibit the growth of epithelial ovarian cancers (1, 10-14). The inhibitory action of [D-Trp[6]]LH-RH on ovarian cancer growth was thought to be mediated mainly by the suppression of the pituitary–gonadal axis, but some of the inhibitory effects could be direct, since human ovarian epithelial cancers have LH-RH-binding sites (1, 3, 7).

The modern LH-RH antagonist [Ac-D-Nal(2)[1], D-Phe(4Cl)[2], D-Pal(3)[3], D-Cit[6], D-Ala[10]]LH-RH [SB-75 (Cetrorelix), in which Ac-D-Nal(2) = N-acetyl-3-(2-naphthyl)-D-alanine, D-Phe(4Cl)-4-chloro-D-phenylalanine, D-Pal(3) = 3-(3-pyridyl)-D-alanine, and D-Cit = D-citrulline] is free of edematogenic and anaphylactoid reactions, and it powerfully inhibits LH secretion and blocks ovulation in rats (15, 16). The use of LH-RH antagonists in cancer therapy would prevent the temporary clinical "flare-up" of disease that occurs initially in response to LH-RH agonists in some malignancies such as prostate cancer (1). During the past few years, various human epithelial ovarian carcinoma cell lines such as OVCAR-3, EFO-21, EFO-27, and OV-1063 were established (6, 7, 17). OV-1063 human epithelial ovarian cancer cell line originated from metastatic papillary cystadenocarcinoma of the ovary stage IV in a 57-year-old woman (17). OV-1063 cells are positive for carcinoembryonic antigen (17).

The purpose of the present study was to investigate the effects of LH-RH antagonist SB-75 and agonist [D-Trp[6]]LH-RH on the growth of xenografts of human epithelial ovarian cancer OV-1063 in nude mice. To shed light on the possible mechanisms of antitumoral action of the analogs, various endocrine and pathological evaluations and measurements of receptors were performed.

MATERIALS AND METHODS

Chemicals. LH-RH antagonist SB-75, originally synthesized in our laboratory by solid-phase methods (15), was made by Asta Medica (Frankfurt am Main, Germany). LH-RH agonist [D-Trp[6]]LH-RH was synthesized by solid-phase methods and supplied by Debiopharm (Lausanne, Switzerland). In the first experiment, SB-75 pamoate microcapsules, lot RCS-ES 9107M, were used. They were prepared by P. Orsolini (Cytotech, Martigny, Switzerland) as described (16) and consisted of SB-75 pamoate [17.9% (wt/wt)] distributed within a polymer matrix of poly(DL-lactide-coglycolide). Microcapsules of [D-Trp[6]]LH-RH in poly(DL-lactide-coglycolide) were also prepared by a phase-separation process by P. Orsolini at Cytotech and supplied by Debiopharm.

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Abbreviations: LH, luteinizing hormone; LH-RH, LH-releasing hormone; IGF-I, insulin-like growth factor I; EGF, epidermal growth factor; D-Nal(2), 3-(2-naphthyl)-D-alanine; D-Phe(4Cl), 4-chloro-D-phenylalanine; D-Pal(3), 3-(3-pyridyl)-D-alanine; Cit, citrulline; NOR, nucleolar organizer region; AgNOR, argyrophilic NOR.

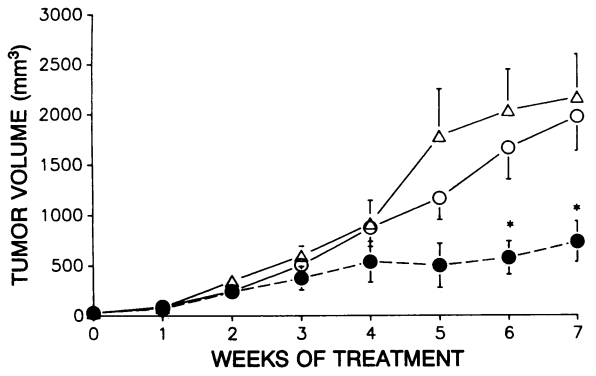

FIG. 1. Tumor volume in nude mice bearing xenografts of OV-1063 human epithelial ovarian cancer during treatment with microcapsules of LH-RH antagonist SB-75 (●) or agonist [D-Trp⁶]LH-RH (△). Vertical lines indicate SEM. *, $P < 0.05$ vs. control by Duncan's new multiple range test. ○, Control.

RPMI 1640 medium and 0.05% trypsin/0.02% EDTA were purchased from GIBCO. Fetal bovine serum (FBS) was obtained from Irvine Scientific. All other chemicals, unless otherwise mentioned, were obtained from Sigma.

Animals. Five- to 6-week-old female athymic NCr *nu/nu* nude mice were obtained from the Frederick Cancer Research Facility of the National Cancer Institute (Frederick, MD). The mice were housed in sterile cages under laminar flow hoods in a temperature-controlled room with a 12-hr light/12-hr dark schedule and were fed autoclaved chow and water *ad libitum*.

Cells and Tumors. OV-1063 cells were kindly provided by the late Shoshana Biran (Hadassah University, Jerusalem) and were maintained in phenol red-free RPMI 1640 medium, supplemented with 10% heat-inactivated and dextran-coated charcoal-treated FBS (DCC-FBS), which was prepared as described (18). The medium also contained 25 nM Hepes buffer, 2 mM glutamine, 100 units of penicillin per ml, 100 μg of streptomycin per ml, and 0.25 μg of amphotericin B per ml. Cells were cultured in Costar T-75 flasks in a humidified atmosphere of 5% CO_2/95% air at 37°C. OV-1063 cells growing exponentially were transferred into nude mice by s.c. injection of 1×10^7 cells into the right flank of five mice. The mice were checked for reproductive cyclicity by vaginal smears. All of the mice bearing growing tumors did not exhibit the usual 4- to 5-day estrous cycle.

Experimental Protocol. In the first experiment, xenografts of 1 mm³ OV-1063 tumor pieces were transplanted subcutaneously into the flanks of nude mice. The treatment with LH-RH analogs was started 25 days after tumor transplantation, when the tumors measured ≈30 mm³, and was continued for 7 weeks. The mice were divided into three groups (six to eight animals per group) and received the following treatments: group 1 (the control), the injection vehicle alone was administered every 4 weeks; group 2, SB-75 microcapsules releasing 60 μg/day were administered every 4 weeks; and group 3, [D-Trp⁶]LH-RH microcapsules releasing 25 μg/day were injected every 4 weeks. Both types of microcapsules were suspended in 0.6 ml of injection vehicle consisting of 2% carboxymethylcellulose and 1% Tween 80 in distilled water. The suspension was thoroughly mixed on a Vortex mixer and injected subcutaneously (s.c.) through an 18-gauge needle on days 1 and 28 of the treatment period.

In the second experiment, the treatment was started 2 weeks after tumor transplantation, when the tumors measured ≈10 mm³ and was continued for 3 weeks. The mice were divided into three groups (10 animals per group). The control group was injected s.c. with 0.9% saline only. Group 2 received SB-75 dissolved in distilled water containing 5% mannitol by daily s.c. injections at a dose of 100 μg/day per animal. Group 3 was administered s.c. [D-Trp⁶]LH-RH in saline at a dose of 100 μg/day per animal.

Tumors were measured weekly with microcalipers, and tumor volume was calculated by using the following formula: length × width × height × 0.5236 (19). Percentage change in tumor volume from the start of the treatment was used as a parameter of growth rate. Tumor doubling time was calculated between the start and the end of the treatment. At the end of the first experiment, the mice were sacrificed under light methoxyflurane (Metofane; Pitman–Moore, Washington Crossing, NJ) anesthesia, and blood was collected from the abdominal aorta. All the blood samples were centrifuged, and serum was stored at −20°C until assayed. Tumors and sex organs (uteri and ovaries) were removed, cleaned, and weighed. Tumor burden at the end of the experiment was calculated as tumor weight (mg)/body weight (g). Pieces of tumor tissue were fixed in 10% buffered neutral formalin for histological examination. Tumor pieces were stored at −80°C for receptor studies.

Determination of Serum LH and Estradiol Levels. Serum LH and estradiol levels were determined as described (20).

Pathological Procedures. Specimens were embedded in Paraplast (Oxford). Step sections 6 μm thick were cut and stained with hematoxylin/eosin. Mitotic and apoptotic cells were counted in 10 high-power fields, and their number per 1000 cells was calculated. For the demonstration of the nucleolar organizer region (NOR) in tumor cell nuclei, the argyrophilic NOR (AgNOR) method of Chiu *et al.* (21) was used with little modification (22). The silver-stained black dots in 50 cells of each tumor were counted and the AgNOR number per cell was calculated. The extent of necrosis was measured on the slide containing the largest cross section of tumors, and the percentage area of necrosis was calculated by using an ocular net with 100 crossing points. All counts were carried out without knowledge of treatment.

Epidermal Growth Factor (EGF) and Insulin-Like Growth Factor (IGF-I) Binding Studies in Tumor Membranes. ^{125}I-labeled EGF (specific activity, 750 Ci/mmol; 1 Ci = 37 GBq) and ^{125}I-labeled IGF-I (2000 Ci/mmol) were purchased from Amersham. Tumor membrane preparation and receptor binding of EGF and IGF-I were performed as described (23, 24). The LIGAND-PC computerized curve-fitting program of Munson and Rodbard (25) was used to determine the types of receptor binding, the dissociation constant (K_d), and the maximal binding capacity of receptors (B_{max}).

Statistical Analyses. All data are expressed as the mean ± SEM, and statistical analyses were performed by using Duncan's new multiple range test or Student's *t* test (16, 20, 22).

Table 1. Tumor, body, and organ weights in nude mice bearing xenografts of OV-1063 human epithelial ovarian cancer cell line after treatment with microcapsules of SB-75 or [D-Trp⁶]LH-RH

Treatment	Body weight, g	Tumor weight, mg	Uterine weight, mg	Ovarian weight, mg
Control	25.8 ± 1.3	1970.4 ± 342.3	59.3 ± 3.7	7.5 ± 0.5
SB-75	28.0 ± 1.1	924.0 ± 308.6*	23.2 ± 2.1*	4.4 ± 0.4**
[D-Trp⁶]LH-RH	26.0 ± 1.7	2075.0 ± 414.9	38.4 ± 2.3*	5.0 ± 0.4**

The results are mean ± SEM. *, $P < 0.05$; and **, $P < 0.01$, both vs. control by Duncan's new multiple range test.

Table 2. Effect of treatment with microcapsules of SB-75 or [D-Trp^6]LH-RH on tumor volume, percentage change in tumor volume, tumor burden, and tumor doubling time in nude mice bearing xenografts of OV-1063 human epithelial ovarian cancer cell line

Treatment	Tumor volume, mm^3 Initial Day 0	Tumor volume, mm^3 Final Day 49	% increase in tumor volume from day 0	Tumor burden, mg/g of body weight	Tumor doubling time, days
Control	31.2 ± 8.4	1974.3 ± 343.0	7529 ± 1200	70.1 ± 11.2	8.0 ± 0.3
SB-75	31.4 ± 5.4	734.1 ± 202.6*	2615 ± 744*	32.4 ± 10.3*	11.5 ± 1.2**
[D-Trp^6]LH-RH	31.0 ± 6.1	2165.6 ± 428.6	8355 ± 1706	100.4 ± 9.1	8.1 ± 0.5

The results are means ± SEM. *, $P < 0.05$, and **, $P < 0.01$ both vs. control by Duncan's new multiple range test.

RESULTS

In the first study, the treatment with LH-RH analogs was started 25 days after tumor transplantation, when the tumors measured ≈30 mm^3, and continued for 7 weeks. The OV-1063 tumors in the control group grew rapidly and continuously throughout the treatment period (Fig. 1). Administration of antagonist SB-75, but not of [D-Trp^6]LH-RH, inhibited the growth of ovarian cancer. At the end of the first experiment, there were no significant differences in body weights between groups, but tumor weights and tumor burden were significantly reduced by administration of SB-75 microcapsules, as compared with the control (Tables 1 and 2). The final tumor volume and the percentage change in tumor volume were also significantly decreased, and tumor doubling time was prolonged in the group treated with SB-75 microcapsules, but not with microcapsules of [D-Trp^6]LH-RH.

In the second experiment, the treatment was started 2 weeks after tumor transplantation, when the tumors measured ≈10 mm^3, and was continued for 3 weeks. Tumor growth in mice receiving SB-75 by daily s.c. injections at a dose of 100 μg/day was significantly reduced (Fig. 2). The final tumor, volume in the group treated with SB-75 was decreased to 158.8 ± 60.4 mm^3 as compared with the controls, which measured 1046.0 ± 66.5 mm^3. Tumor weights were reduced by ≈85% as compared with the control groups (0.13 ± 0.05 g vs. 0.86 ± 0.1 g in controls) after 3 weeks of treatment with SB-75. Therapy with LH-RH agonist [D-Trp^6]LH-RH administered by daily s.c. injections at a dose of 100 μg/day had no significant effect on tumor volume (Fig. 2) or tumor weights.

Uterine and ovarian weights were decreased in mice treated with both analogs in the first experiment (Table 1). As shown in Table 3, serum LH levels of the control animals were higher than those of cyclic nude mice without OV-1063 tumors and were suppressed by administration of SB-75 and [D-Trp^6]LH-RH microcapsules. Serum estradiol levels were undetectable in untreated nude mice bearing ovarian OV-1063 tumors and treated groups. Similar results were obtained in the second study.

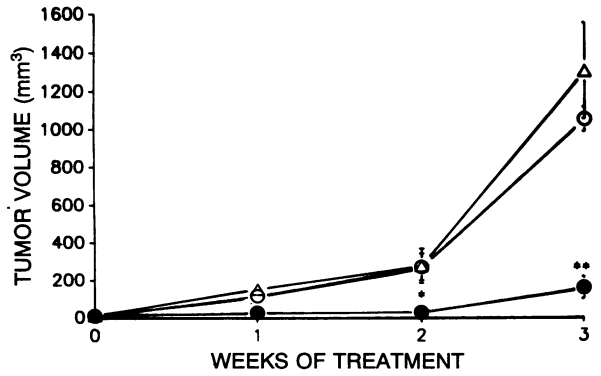

FIG. 2. Tumor volume in nude mice bearing xenografts of OV-1063 human epithelial ovarian cancer during treatment with LH-RH antagonist SB-75 (●) or agonist [D-Trp^6]LH-RH (△). Both analogs were administered by daily s.c. injections in doses of 100 μg per animal. Vertical lines indicate SEM. ○, Control; *, $P < 0.05$, and **, $P < 0.01$, both vs. control by Duncan's new multiple range test.

Histologically, the tumors were nondifferentiated adenocarcinomas. The histological appearance of tumors was similar in all groups. The treated tumors did not differ from the control tumors in the extent of necrosis, the number of mitotic or apoptotic cells (22), and the number of AgNORs (22) (Table 4). The finding that there were no significant histological differences between the SB-75-treated and untreated tumors, despite the inhibition of tumor growth, might be explained by the fact that blood levels of the LH-RH analogs were likely to be low when the experiment was terminated (16) and the number of mitotic and apoptotic cells or AgNORs in tumors can rapidly return to original levels (22).

The results of receptor assays in the first experiment are shown in Table 5. High-affinity binding sites for both EGF- and IGF-I were present in cell membranes of the control tumors. A significant reduction in EGF and IGF-I binding capacity was observed after treatment with SB-75. B_{max} of IGF-I binding sites was also significantly decreased by administration of [D-Trp^6]LH-RH, but B_{max} of EGF binding sites was increased.

DISCUSSION

The present study demonstrates that LH-RH antagonist SB-75 administered once a month in the form of sustained-release microcapsules or by daily injections inhibits the growth of OV-1063 human epithelial ovarian cancer xenografts in nude mice. The inhibition of tumor growth by administration of SB-75 was accompanied by a marked suppression of pituitary and gonadal functions.

Treatment with LH-RH agonists or antagonists leads to selective medical hypophysectomy and chemical castration and provides an efficacious approach for the treatment of some hormone-dependent tumors such as prostate cancers and breast cancers (1, 20, 26, 27). LH-RH agonists including [D-Trp^6]LH-RH have been used for the treatment of women with epithelial ovarian cancer (10, 12-14), but in a study involving 41 patients with advanced ovarian cancer, the response rate was only about 15% (28). The reduction in blood levels of gonadotropins and sex steroids, induced by

Table 3. Serum LH and estradiol levels in nude mice with xenografts of OV-1063 human epithelial ovarian cancer cell line after treatment with microcapsules of SB-75 or [D-Trp^6]LH-RH

Treatment	LH, ng/ml	Estradiol, pg/ml
Control	0.36 ± 0.03	ND
SB-75	0.18 ± 0.04**	ND
[D-Trp^6]LH-RH	0.27 ± 0.01*	ND
Normal nude mice (without tumors)	0.26 ± 0.04*	2.15 ± 0.19

The results are means ± SEM. *, $P < 0.05$, and **, $P < 0.01$ both vs. control by Student's t test. ND, not detectable by RIA.

Table 4. Effect of treatment with microcapsules of SB-75 or [D-Trp^6]LH-RH microcapsules on histological characteristics of OV-1063 human epithelial ovarian cancer xenografted in nude mice

Treatment	% area of necrosis	No. of mitoses per 1000 cells	No. of apoptotic cells per 1000 cells	No. of AgNORs per nucleus
Control	18.8 ± 4.6	7.4 ± 1.1	7.3 ± 1.1	10.2 ± 0.3
SB-75	27.2 ± 11.5	7.9 ± 2.1	8.9 ± 1.0	9.5 ± 0.3
[D-Trp^6]LH-RH	39.4 ± 12.4	5.4 ± 0.6	9.5 ± 1.6	10.4 ± 0.4

The results are means ± SEM.

LH-RH analogs, may be useful for the treatment of ovarian cancer. High levels of gonadotropins in women after menopause have been implicated in ovarian carcinogenesis (1, 3–8). The growth of some ovarian cancer cell lines was stimulated by gonadotropins (8). However, in preliminary studies, we found that human chorionic gonadotropin did not enhance the growth of the OV-1063 cell line (29). Similarly, Pergonal, a human gonadotropin preparation did not stimulate the proliferation of human epithelial ovarian cancer cell line 2774 (30). In the present study, the nude mice bearing growing ovarian OV-1063 tumors were all noncyclic, and their serum estradiol levels were undetectable. Serum LH level of control nude mice with tumors were higher than those of cyclic mice. Although [D-Trp^6]LH-RH decreased serum LH levels significantly, it did not inhibit tumor growth in this ovarian cancer model. Thus, the role of gonadotropins in the growth of OV-1063 cells needs to be investigated further.

In our recent *in vitro* study (29), the growth of OV-1063 cells was inhibited at physiologic concentrations of estradiol, but this inhibition was not found at extremely high and low levels of estradiol, which suggests that the growth of ovarian cancers could be enhanced under abnormal endocrine conditions. These observations are in agreement with other results (31–33) and with the report that a decreased incidence of ovarian cancer was observed in women who underwent estrogen replacement therapy during menopause (5). We also showed that SB-75 and [D-Trp^6]LH-RH can suppress the growth of OV-1063 cells *in vitro* directly through high-affinity LH-RH receptors under estrogen-deprived conditions (29). Recently, Emons *et al.* (7) demonstrated the presence of high-affinity LH-RH binding sites in EFO 21 and EFO 27 human epithelial ovarian cancer lines and showed that both the agonist [D-Trp^6]LH-RH and antagonist SB-75 inhibited the proliferation of these cell lines *in vitro*. Collectively, these findings (7, 29) support the view that the antitumor effect of SB-75 in the present study could have been exerted in part directly through LH-RH receptors. However, we do not have a clear explanation why under the conditions of our *in vivo* study [D-Trp^6]LH-RH failed to inhibit the growth of OV-1063 tumors in nude mice. There can be several reasons for the lack of effect of [D-Trp^6]LH-RH. The inhibitory effect of SB-75 on growth of OV-1063 cell line *in vitro* was greater than that of [D-Trp^6]LH-RH, the cell proliferation being suppressed by SB-75 at a concentration of 10 μM, but not by 10 μM [D-Trp^6]LH-RH (29). Thompson *et al.* (30) also reported that antiproliferative effects of LH-RH agonist leuprolide on human epithelial ovarian cancer cell line 2774 were observed at concentrations >10 μM (30). It is possible that OV-1063 cell line has a LH-RH antagonist binding site that is not recognizable by LH-RH agonists, as previously reported for MCF-7 human breast cancer cell line (34). It is also likely that there could be a difference in the mechanism of antiproliferative effect at the receptor level between LH-RH agonists and antagonists. Eidne *et al.* (35) reported previously that [^{3}H]thymidine incorporation in human breast cancer cell lines was inhibited by LH-RH antagonists, but not by LH-RH agonists.

There is also evidence that EGF and IGF-I may play an important role in the regulation of proliferation of human ovarian cancer cells (33, 36-39). High-affinity binding sites for EGF and IGF-I have been identified in human epithelial ovarian cancer tissue (36-39). Particularly, EGF receptor-positive status was shown to be correlated with the biological aggressiveness of ovarian cancer (36, 37). Previously, we have shown that inhibition of growth of estrogen-dependent MXT tumors in mice by SB-75 and [D-Trp^6]LH-RH was associated with a reduction in the concentration (down-regulation) of EGF receptors in tumor membranes (40). Our present study indicates that SB-75 induced a significant down-regulation of EGF and IGF-I receptors also in OV-1063 cells. In contrast, [D-Trp^6]LH-RH caused an increase in the number (up-regulation) of EGF receptors. Thus, SB-75 may be acting locally by reducing the available binding sites for EGF on ovarian cancers. This hypothesis has to be confirmed by additional studies, but it would explain the efficacy of antagonist SB-75 in reducing ovarian cancer growth, in contrast to a relative lack of effect of agonist [D-Trp^6]LH-RH. In various experimental studies in several models of breast and prostate cancer, the antagonist SB-75 appeared to be more potent in inhibiting tumor or cancer cell growth than LH-RH agonists (20, 26, 27, 34, 40-42). Recently, Manetta *et al.*‡ also showed that SB-75 inhibited the growth of UC1107 human epithelial ovarian carcinomas in nude mice.

In conclusion, our studies indicate that the growth of OV-1063 human epithelial ovarian cancer xenografts in nude mice can be inhibited by modern LH-RH antagonist SB-75 (Cetrorelix) but not by agonist [D-Trp^6]LH-RH. LH-RH antagonists such as SB-75 (Cetrorelix) might prove to be superior to LH-RH agonists in the treatment of advanced

‡Manetta, A., Gamboa-Vujicic, G., Paredes, P., Emma, D., Leong, L., Asch, B. & Schally, A. V., Pacific Coast Fertility Society Meeting, April 20–24, 1994, Indian Wells, CA, pp. A6–A7 (abstr.).

Table 5. Characteristics of receptors for EGF and IGF-I in membranes of OV-1063 human epithelial ovarian cancer xenografts in nude mice after treatment with microcapsules of SB-75 and [D-Trp^6]LH-RH

	EGF		IGF-1	
Treatment	K_d, nM	B_{max}, pmol/mg of protein	K_d, nM	B_{max}, pmol/mg of protein
Control	7.3 ± 0.5	0.9 ± 0.13	6.3 ± 0.7	0.9 ± 0.08
SB-75	4.8 ± 0.8**	0.5 ± 0.14*	4.6 ± 0.5*	0.4 ± 0.04**
[D-Trp^6]LH-RH	7.4 ± 0.6	1.2 ± 0.11*	5.6 ± 0.5	0.5 ± 0.06**

Values are means ± SEM. The results were obtained from 10-point displacement experiments in triplicate tubes. *, $P < 0.05$, and **, $P < 0.01$, both vs. control by Duncan's new multiple range test.

ovarian epithelial carcinomas (1). Treatment of epithelial ovarian cancer with LH-RH antagonists could offer a nontoxic alternative in patients who do not tolerate chemotherapy or who have progressive disease following chemotherapy. In view of its powerful inhibitory effect on OV-1063 tumors and lack of side effects, SB-75 (Cetrorelix) could be considered for the treatment of advanced epithelial ovarian carcinomas.

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