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INFLUENCE OF LUTEINIZING HORMONE-RELEASING HORMONE AGONISTS ON HUMAN MAMMARY CARCINOMA CELL LINES AND THEIR XENOGRAFTS

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Summary—The specific binding of luteinizing hormone-releasing hormone (LH-RH) agonist in estradiol-dependent MCF-7 and estradiol-independent MDA-MB-231 human breast cancer cells has been studied using [^{3}H]Ovurelin [(D-^{3}H-Phe^6),des-Gly^{10}-LH-RH-ethylamide]. The results of Scatchard analyses suggest the presence of a single class of receptor sites, both in cell suspensions and membrane fractions. Evaluation of these peptide receptors appears to reflect additional characteristics of biological behaviour of these human breast cancer cells. The synthetic LH-RH agonist Ovurelin [(D-Phe^6),des-Gly^{10}-LH-RH-ethylamide] can directly interfere (25–30%) with the proliferation of MDA-MB-231 human breast cancer cells in culture. The inhibitory effect of Ovurelin *in vitro* was negligible in the MCF-7 cell line.

In the *in vivo* experiments the treated immunosuppressed mice bearing either MCF-7 or MDA-MB-231 xenografts responded to the high-dose LH-RH analogue Zoladex depot and Decapeptyl depot therapy. Since the MDA-MB-231 tumour was found to be ER-negative it seems possible that the regression of this xenograft results from the direct antitumour action of the LH-RH agonist.

INTRODUCTION

Chronic administration of potent agonists of LH-RH results in the inhibition of pituitary and gonadal function. Due to the desensitization of cells in the hypophysis, the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) is suppressed. This enables LH-RH agonists to elicit a chemical castration-like response, while producing and maintaining castrate concentrations of sex steroids in the plasma, making them suitable for the treatment of estrogen-dependent breast cancer [1–4]. Although the antitumour effect of LH-RH analogue on breast cancers is thought to be due primarily to the creation of a state of estrogen deficiency [5–8], recent receptor-binding studies have revealed that LH-RH analogues can also exert direct effects on certain breast tumours [5–9]. The inhibitory effects of LH-RH antagonists point to the possibility of an autocrine regulatory role of LH-RH-like peptides in mammary cells [6]. A new rational combination of endocrine therapy for breast cancer might be based not only on the status of sex-steroid hormone receptors but also on the presence of receptors for different peptides.

The exact mechanism of the direct and indirect actions of LH-RH analogues is not yet completely understood. The aim of this study was to investigate the influence of LH-RH agonists treatment on estradiol-dependent, estradiol receptor (ER)-positive MCF-7 and ER-negative MDA-MB-231 human breast cancer cell lines and xenografts. The effects of treatment with LH-RH agonists on ER and progesterone receptor (PR) content in MCF-7 cells were also studied.

MATERIALS AND METHODS

Reagents

[3-^{125}I]iodotyrosyl^5-LH-RH and [^{3}H]ORG 2058 (16-α-ethyl-21-hydroxy-19-nor[6,7-^{3}H]-pregn-4-en-3,20-dione) with specific activities of 74 and 2.15 TBq/mmol, respectively, were purchased from Amersham, U.K. 17-β-[2,4,6,7-^{3}H]-estradiol specific activities of 2.9 TBq/mmol (Hungarian Academy of Sciences, Institute of

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Isotopes, Budapest) was used. Unlabelled LH-RH was obtained from Calbiochem. Ovurelin [(D-Phe^6),des-Gly^{10}-LH-RH-EA] and [^{3}H]-Ovurelin [(D-^{3}H-Phe^6],des-Gly^{10}-LH-RH-ethylamide] were synthesized in the Semmelweis University Medical School (SOTE) [10], Budapest, Hungary. All other chemicals were of analytical grade. Decapeptyl depot (D-Trp^6-LH-RH microcapsule) was kindly provided by Ferring Arzneimittel GmbH, Kiel.

[^{3}H]Ovurelin [(D-^{3}H-Phe^6),des-Gly^{10}-LH-RH-ethylamide] syntheses

(D-^{3}H-Phe^6),des-Gly^{10}-LH-RH-ethylamide (I) and [^{3}H]Ovurelin were synthesized, as published earlier [10] from *p*-chloro-D-Phe^6,des-Gly^{10}-LH-RH-ethylamide (II). 4 mg II was dissolved in 1 ml dimethyl-formamide, 0.01 ml triethylamine was added to the solution then 25.7 mg 10% Pd on $BaSO_4$ catalyst. The mixture was reacted with tritium gas in a special vacuum manifold. Tritium uptake stopped in 120 min. Solvent was removed *in vacuo*, then 2 M acetic acid solution was distilled from the residue several times. The residue was dissolved in 2 M acetic acid solution and spotted on a silica gel plate (Merck, 5721DC-Fertigplatten Kieselgel 60). TLC was developed by *n*BuOH–EtOAc–H_2O–AcOH = 1:1:1:1 solution. TLC was tested by Medifort-RP-Film (Forte-Photochemical Industry, VAC, Hungary) and a narrow strip on TLC with Erlich reagent. Silica gel containing the pure I was removed from the plate, then it was extracted with 2 M acetic acid solution. Solvent was removed *in vacuo*, residue was dissolved in water. 1.25 μM (1.63 mg) I ([^{3}H]Ovurelin) was recovered (estimated by u.v. absorption). Specific radioactivity of I was 6.3 Ci/mmol = 233 GBq/mmol. Radiochemical purity of I was checked by HPLC conditions: C-18 BST column. Elution 38%, CH_3CN 62%, 0.1% TFA-H_2O (Isocratic). Flow rate: 1 ml/min, TR = 7.2 min. After storing I for 1 yr in liquid N_2 about 25% decomposition was observed, therefore the contaminated material was repurified using HPLC methodology in the conditions mentioned above.

Cell cultures

In the experiments, MCF-7 and MDA-MB-231 human breast cancer cell lines were used. The cell lines originated from Dr Jan Kovaric (Res. Inst. Clin. Expl. Oncol., Brno, Czechoslovakia). The cells were grown in plastic flasks (Greiner) in Dulbecco's modified Minimal Essential Medium (DMEM, Gibco) supplemented with 10% newborn calf serum (Phylaxia, Hungary), 1 μg/ml of insulin and 1 μg/ml of Na_2SeO_3. Cells were subcultured twice a week and kept in an atmosphere of 5% CO_2 in air at 37°C. Three weeks before the experiments, the MCF-7 cells were adapted to a medium depleted of estrogens by treatment of the serum with dextran-coated charcoal (DCC), according to the method of Eckert *et al.* [11] and the serum concentration was lowered to 5%.

Xenografts

The xenografts from MCF-7 and MDA-MB-231 human breast cancer cell lines were established in 8-wk-old immunosuppressed CBA/Ca female mice by inoculating either 2×10^7 MCF-7 or 2×10^7 MDA-MB-231 cells s.c. (Immunosuppression: thymectomy of 6-wk-old animals; ^{60}Co whole body irradiation with 9.50 Gy lethal dose; syngene bone marrow transplantation within 24 h.) Further passages were made using implanted tumour pieces (2 mm^3). The mice bearing MCF-7 xenografts were treated s.c. with 50 μg Estradiol Valerate and 30 μg Norgestomet (Intervet International) once a week which were necessary for tumour take and stimulation. The steroid hormone treatment was finished 6 wk after transplantation (at least 1 wk before ER determination). The xenografts were frozen and stored in liquid nitrogen immediately after excision.

Progesterone receptor (PR) induction in MCF-7 cells

Cells (1×10^6) were seeded in a medium supplemented with 5% DCC-FCS in 8 T-175 flasks containing 10 nM 17-β-estradiol (E_2) for PR induction. One day later, the medium was replaced by fresh medium containing either solvent (0.1% ethanol) or 10 nM concentration of E_2. The medium was renewed every day for 6 days. In each of our experiments the medium from near-confluent T-175 flasks of cells was decanted, and cells were harvested with a rubber policeman. The flasks were rinsed with PBS, and the cells were pelleted at 300 ***g*** for 5 min. Cells were transferred to Dounce homogenizer with cold 5 × TEG buffer (50 mM Tris-HCl, 7.5 mM EDTA and 10% glycerol, pH 7.4) containing 1 mM phenylmethylsulfonylfluoride and then homogenized. The cytosol PR contents were determined according to Nardulli *et al.* [12]. The protein concentration of the cytosol was determined by Bio-Rad protein assay or according to Lowry *et al.* [13].

Determination of cytosol ER (cER) and nuclear ER (nER) in cell lines and in their xenografts

The preparation of cytosol and nuclear extract from cell lines and determination of cER and nER contents were performed according to Reiner *et al.* [14] with some modifications. The TETG-KCl (0.8 M KCl) buffer extract from the nuclear pellet was centrifuged at 100,000 *g* for 45 min. An "exchange" method using six concentrations of [^{3}H]17β-E_2 (10–1 nM) with or without a 200-fold excess of unlabelled diethylstilbestrol (DES) was performed. The nER[^{3}H]-17β-E_2 complexes were separated by hydroxylapatite. The binding parameters were estimated by multipoint Scatchard analysis [16]. The protein content of the cytosol was analyzed by the method of Lowry *et al.* [13]. For the determination of DNA content of the nuclear pellet, the Burton's method was applied [15]. Tumour specimens from the xenografts were minced and pulverized by a homogenizer (B. Braun, Melsungen AG) and homogenized in ice-cold TETG buffer (Tris-HCl 10 mM, EDTA 0.5 mM, tioglicerin 12 mM, glicerin 10%, pH = 7.4) by a Dounce homogenizer at 4°C. Cytosol ER and PR binding capacities of the tumour were determined according to the recommendation of EORTC Breast Cancer Cooperative Group [17]. The binding sites and kinetic parameters were calculated by multipoint Scatchard analysis [16]. The preparation of the high-speed nuclear extract from the nuclear pellet was made by the method described above.

LH-RH binding assay in cell suspensions

The method described by Miller *et al.* [9] was used with some modifications. Confluent cell cultures in T-175 flasks were washed with PBS, precipitated by centrifugation. About 4–5 × 10^7 cells were resuspended in 12 ml Hanks solution containing 20 mM HEPES and 0.25 mg/ml BSA. Aliquots (about 1 × 10^6 cells) of cell suspensions were then incubated in polypropylene tubes together with [^{3}H]Ovurelin (sp. act. 6.3 Ci/mmol) in the presence or absence of a 200-fold excess of non-labelled Ovurelin. (All assay tubes were prewashed with 2% BSA.) Ten different concentrations of labelled peptide were used for radioreceptor assay. The cells were incubated for 60 min at 4°C. After addition of 2 ml ice-cold medium per tube the cells were precipitated by centrifugation at 4°C for 5 min at 15,000 *g*. The pellets were washed and spun down again, then transferred into scintillation vials. The samples were analyzed in a Beckmann LS 9800 Liquid Scintillation system. The binding parameters were calculated by multipoint Scatchard analysis.

LH-RH binding to membrane fraction of human breast xenografts

The separation of membrane fraction and the binding studies were previously described by Marshall and Odell [18]. Various concentrations of tritiated LH-RH agonist [(D-^{3}H-Phe^6), des-Gly^{10}-LH-RH-ethylamide, Ovurelin] were incubated with 300 μl of membrane suspension in the presence or absence of 200-fold excess of unlabelled Ovurelin. After incubation at 4°C for 60 min, the membrane-bound LH-RH was separated by centrifugation at 15,000 *g* and radioactive concentrations were measured. The protein content of membrane suspension was determined by a Bio-Rad protein assay.

RESULTS

Steroid receptor (cER, nER, cPR) content of MCF-7 cell line and xenografts

In agreement with findings of others [11, 12] the MCF-7 cells and xenograft were ER-positive (Table 1) while the MDA-MD-231 cells and tumours were ER-negative. The basal cPR level of cells was about 10 fmol/mg protein or below (Table 1). For the development of the MCF-7 xenograft in immunosuppressed mice, E_2 was found to be the most potent stimulator of the tumour growth. In the first 6 wk the tumours could not grow without exogeneous E_2 treatment. Six weeks after the implantation, however, other autocrine and/or paracrine growth factors and the endogeneous E_2 concentration seemed to be sufficient to maintain the xenograft's growth.

Table 1. Steroid receptor contents in human breast cancer cell line and xenografts

Passages	Time after transplantation (wk)	cER (fmol/mg protein)	nER (pmol/mg DNA)	cPR (fmol/mg protein)
Cell line (5% DCC-FCS)		56 ± 10 (25)[a]	2.5 ± 1 (25)	10 ± 5 (15)
P_0 (5)[a]	14	51 ± 8	2.5 ± 0.8	12 ± 5
P_1 (5)	10	53 ± 7	3.0 ± 0.4	18 ± 6
P_1 (5)	12	50 ± 7	3.5 ± 0.2	20 ± 8
P_2 (5)	7	59 ± 9	2.7 ± 0.4	21 ± 7
P_4 (3)	6	54 ± 10	2.9 ± 0.5	25 ± 5
P_4 (3)	10	49 ± 6	3.0 ± 0.6	20 ± 6
P_6 (3)	6.5	58 ± 8	2.2 ± 0.8	22 ± 8
P_6 (3)	9	55 ± 8	2.9 ± 0.6	18 ± 4
P_6 (3)	13	54 ± 9	3.2 ± 0.8	22 ± 7
P_6 (3)	15	50 ± 6	3.5 ± 0.6	24 ± 5
P_7 (5)	6.5	51 ± 7	3.3 ± 0.8	19 ± 6

Results are given as means ± SE.
[a]Numbers in parentheses indicate number of determinations.

Table 2. *In vitro* effect of a 6-day 10 nM 17-β-E_2 treatment on MCF-7 cell line

Treatment	Protein concentration per flask (mg)	Cell number per flask ($\times 10^6$)
Control	2.4 ± 0.3 (9)[a]	7.3 ± 0.6 (9)
10 nM 17-β-E_2	4.9 ± 0.4 (9) ($P < 0.05$)	14.5 ± 0.7 (9) ($P < 0.05$)

Results are given as means ± SE.
[a]Numbers in parentheses indicate number of determinations.
P-value was determined by Student's *t*-test.

The length of the latent phase and the growth rate of the tumour showed no change from passage 4. No significant change was observed in the cER, nER or cPR contents of MCF-7 tumours by the age of the xenografts (Table 1). Dissociation constants (K_d) for specific binding of E_2 (in cytosol and nuclear fractions) and progesterone (in cytosol fraction) to their receptor protein were in the range of $K_d = 0.1$–0.2×10^{-9} M.

Estradiol sensitivity of MCF-7 cell line

The MCF-7 cell line is heterogeneous, containing several subpopulations, so the cER, nER and cPR contents are different. The mitogenic effect of 10 nM 17-β-E_2 on the growth of MCF-7 (medium containing lower than 5% DCC-FCS) was significant in response to a 6-day treatment (Tables 2 and 3), in agreement with the results of Najid *et al.* [19]. After E_2 stimulation, the protein content and the cell number increased two-fold (Table 2) and the basal concentrations of cPR and nER also increased significantly ($P < 0.05$) (Table 3).

Binding studies of (D-^{3}H-Phe^6),des-Gly^{10}-LH-RH-ethylamide

Binding studies were performed in suspension of MCF-7 and MDA-MB-231 cells and in membrane fractions of their xenografts. Both MCF-7 and MDA-MB-231 cells possess specific binding sites for LH-RH agonist (Table 4). The results of multipoint Scatchard analyses suggest that the labelled peptide analogue was bound to a single class of receptor sites both in cell suspensions and membrane fractions with low affinity ($K_d = 33.35 \pm 3.66 \times 10^{-9}$ M) and high capacity (B_{max} of receptors = 3.56 ± 0.33 pmol/mg of membrane protein in the MCF-7 tumour and 4.23 ± 0.39 pmol/mg protein in the membrane fractions of MDA-MB-231 cells). The MDA-MB-231 cells contain a higher amount of receptors for Ovurelin than MCF-7 cells (Table 4).

Table 4. Comparison of binding characteristics of [^{3}H]Ovurelin receptors in cell suspensions and in membrane fractions of mammary cancer samples

Samples	Dissociation constant ($K_d \times 10^{-9}$ M)	[^{3}H]Ovurelin binding capacity (B_{max}) (pmol/mg protein)
MCF-7 cell suspension	36.48 ± 2.80	3.95 ± 0.38
MDA-MB-231 cell suspension	37.12 ± 2.70	4.62 ± 0.41
Membrane fractions of MCF-7 xenograft	30.22 ± 2.10	3.56 ± 0.33
Membrane fractions of MDA-MB-231 xenograft	30.12 ± 2.10	4.23 ± 0.39

Binding characteristics were evaluated from 10-point Scatchard analysis. Results are given as means ± SE.

In vitro effect of (D-Phe^6),des-Gly^{10}-LH-RH-ethylamide

MCF-7 and MDA-MB-231 cells were trypsinized and 2×10^6 and 1×10^6 cells, respectively, were seeded in 16 T-175 flasks in complete medium to allow attachment of the cells to the culture flasks. One day later the medium was changed for the experimental medium, which was renewed daily during a 6-day period both in the control and in the treated flasks with 1 μM Ovurelin. According to the literature, LH-RH agonists are decomposed in the cell culture medium in 24 h, so it was necessary to add Ovurelin each day. By this way we maintained a constant high level of agonist similar to the use of LH-RH agonist depot preparation *in vivo*. One day after a 6-day treatment the changes in protein contents and cell number were measured in both cell lines. In the case of the MCF-7 cell line the cER, nER, cPR concentrations and [^{3}H]Ovurelin binding capacity of the membrane

Table 3. *In vitro* effect of a 6-day 17-β-E_2 or Ovurelin treatment on the cER, nER, cPR and Ovurelin receptor binding capacity of MCF-7 cell line

Treatment	cER (fmol/mg protein)	nER (pmol/mg DNA)	cPR (fmol/mg protein)	[^{3}H]Ovurelin binding (pmol/mg protein)
Control (9)[a]	56 ± 8	2.49 ± 0.4	10 ± 5	3.95 ± 0.38
10 nM E_2 (9)	60 ± 10	9.24 ± 1.2 ($P < 0.05$)	190 ± 24 ($P < 0.05$)	3.23 ± 0.30
1 μM Ovurelin (9)	48 ± 6	3.11 ± 0.8	10 ± 5	1.2 ± 0.10 ($P < 0.05$)

Results are given as means ± SE.
[a]Numbers in parentheses indicate number of determinations.
P-value was determined by Student's *t*-test.

fractions were also measured before and after the treatment.

The 6-day treatment with Ovurelin caused no changes in protein content (control = 4.86 ± 0.42 mg, treated = 4.65 ± 0.32 mg per flask) and in cell number (control = 15.84 ± 0.63 × 10^6, treated = 14.85 ± 0.43 × 10^6 cells per flask) of the MCF-7 line. A 30% decrease ($P < 0.05$) was observed in MDA-MB-231 cells in all parameters compared with the untreated control cells (control = 6.68 ± 0.54 mg, treated = 4.64 ± 0.43 mg protein per flask and control = 20.46 ± 0.86 × 10^6, treated = 14.42 ± 0.53 × 10^6 cells per flask). According to our results no significant change was observed in the cER, nER or cPR content of MCF-7 cells after a 6-day Ovurelin treatment (Table 3). The daily administration of 1 μM Ovurelin significantly decreased ($P < 0.05$) the concentrations of receptors for [^{3}H]Ovurelin (Table 3).

In vivo effect of LH-RH analogue on human xenograft-bearing mice

Preclinical experiments with three different LH-RH agonists were carried out on immunosuppressed mice bearing either MCF-7 or MDA-MB-231 tumours.

A single s.c. depot of Zoladex [D-Ser(tBu)^6, AzGly^{10}, LH-RH ICI 118630] containing 100 μg agonist (liberating 3.5 μg/day), or a single s.c. depot Decapeptyl (D-Trp^6-LH-RH-microcapsule, Ferring Arzneimittel GmbH, Kiel) containing 100 μg peptid analogue (liberating 3.5 μg/day) was given to mice three times at 28-day intervals. At the beginning of treatment with Zoladex the tumours were 46-day old (about 500 mg), while at the beginning of Decapeptyl treatment the tumours were 60-day old (about 750 mg).

One μg Ovurelin (D-Phe^6,des-Gly^{10}-LH-RH-ethylamide, Reanal, Budapest) was injected i.p. twice per day per animal until death of the animals. At the beginning of the administration the tumours were 46-day (Ovurelin I) or 57-day (Ovurelin II) old, and tumour weights were 500 and 750 mg, respectively.

The control animals were not treated at all.

In the case of Ovurelin, in the Decapeptyl depot, control and ovariectomized control animals cER, nER and cPR binding capacities were determined from the non-necrotic part of the tumours on the 7th, 14th and 28th days of administrations, respectively.

The changes of MCF-7 ER-positive tumour weight are demonstrated in Fig. 1. Four to five weeks after transplantation the xenografts were palpable. The tumour growth was stopped by Zoladex or Decapeptyl depot irrespective of the initial tumour weight after 3–4 wk. In the case of Decapeptyl depot treatment, after a slight transient elevation in cER and nER level of the tumours by the 7th day, all steroid receptor binding capacities decreased to the level that was measured in the ovariectomized controls (Fig. 2). Later, the weight of xenografts decreased permanently and the decrease in the tumour size

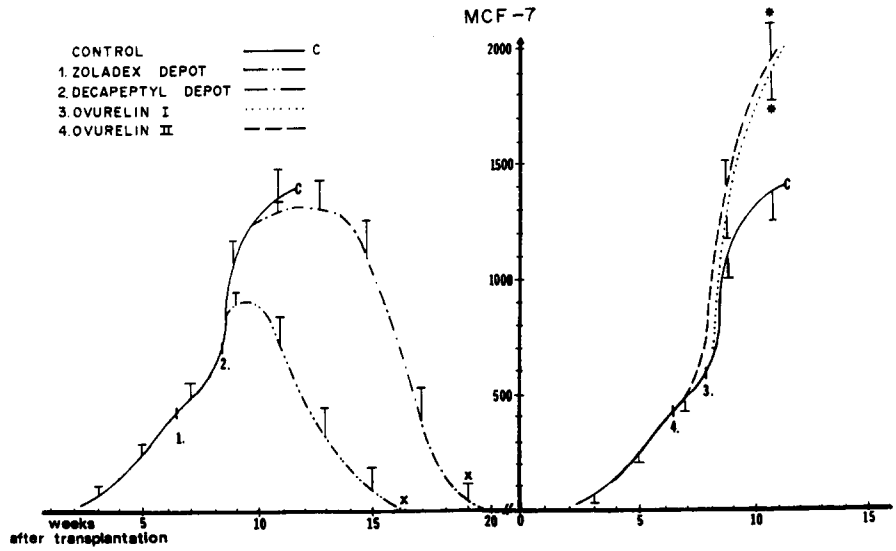

Fig. 1. Right: *in vivo* effect of daily Ovurelin I, II treatment on the weight (mg) of MCF-7 xenografts. Left: *in vivo* effect of Zoladex depot and Decapeptyl depot treatment on the weight of MCF-7 xenografts. Results are given as means ± SE. Significance vs control was calculated with Student's *t*-test ($^{\times}P < 0.01$; $^{*}P < 0.05$).

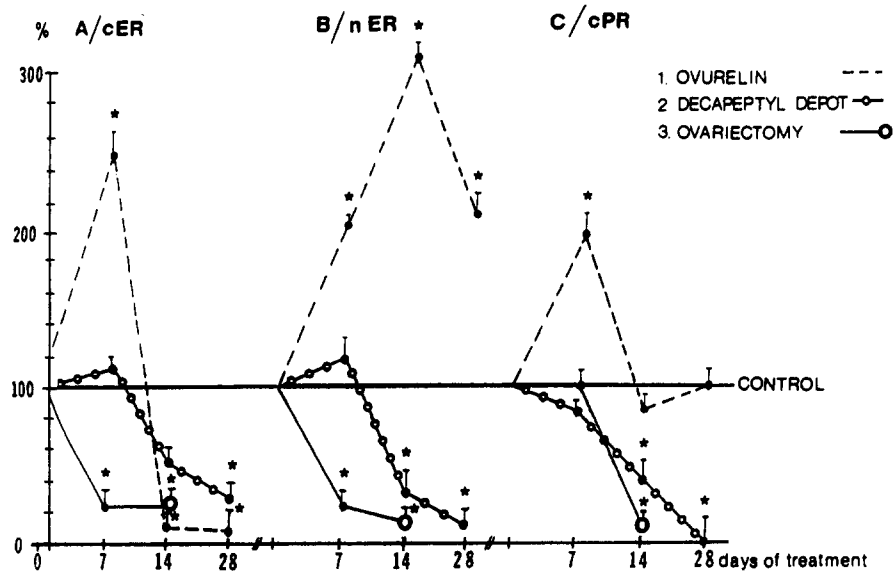

Fig. 2. The changes of cER (A), nER (B) and cPR (C) concentrations of MCF-7 xenografts as compared to the control (100%) on the 7th, 14th and 28th days of Ovurelin or Decapeptyl depot administrations and in the case of ovariectomized control animals. Each determination was made in a group of six animals. Results are given as means ± SE. Significance vs control was calculated with Student's *t*-test (*$P < 0.05$).

was at least 75% ($P < 0.01$), or even the tumour disappeared by the end of the third 28-day interval of Zoladex or Decapeptyl treatment.

The daily i.p. administration of Ovurelin I, II —independent of the initial tumour weight— had a stimulatory effect on the tumour growth ($P < 0.05$) (Fig. 1). Contrary to Decapeptyl depot treatment in the case of Ovurelin administration the cytosol ER, nER, cPR contents of the non-necrotic part of the xenografts increased at the 7th day of the Ovurelin treatment as compared to the control (Fig. 2). By days 14 and 28 the nER content remained elevated, the cPR concentrations decreased to the control level, while the cER could hardly be detected (Fig. 2).

Changes in the weight of the MDA-MB-231 tumour are shown in Fig. 3. The effect of Zoladex and Decapeptyl depot treatment was very similar to that seen in ER-positive MCF-7 xenografts. By the end of the third 28-day

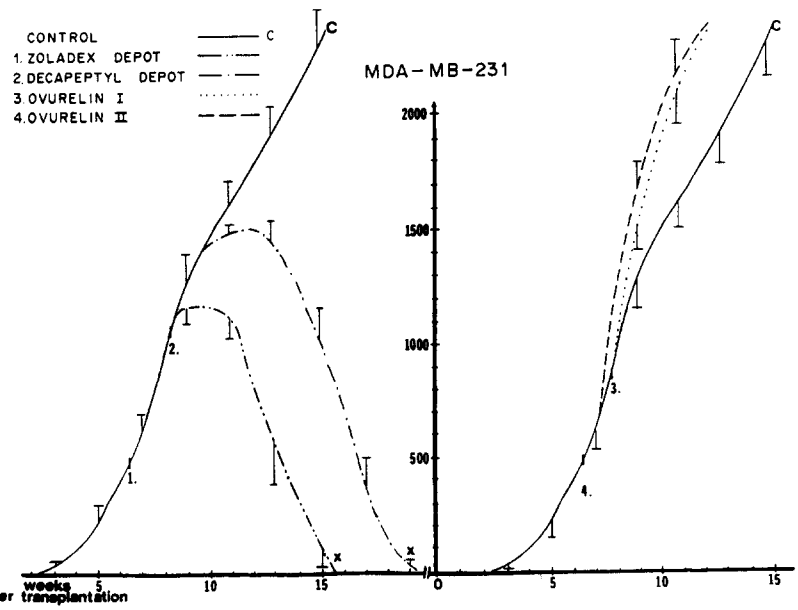

Fig. 3. *In vivo* effect of LH-RH treatments on the weight (mg) of MDA-MB-231 xenografts. Right: Ovurelin I, II administrations. Left: Zoladex depot and Decapeptyl depot treatment. Results are given as means ± SE. Significance vs control was calculated with Student's *t*-test (*$P < 0.01$).

treatment period of Zoladex and Decapeptyl depot administration, the tumour decreased by at least 80% ($P < 0.01$) or it disappeared. The hair of the animals got polished, their body weight increased, and they were in a better condition than the treated MCF-7 xenograft-bearing mice.

It is of interest that the daily Ovurelin treatment also stimulated the ER-negative tumour growth, but this was not significant. No evidence of steroid receptors was found on days 7, 14 and 28 of treatment, similar to controls.

It should be emphasized that the necrotic areas in both types of xenografts increased significantly during the daily Ovurelin administration (data not shown).

DISCUSSION

Clinical studies have shown that certain metastatic breast cancers are responsive to chronic administration of LH-RH analogues. These peptides exert their antitumour effect through direct and indirect mechanisms [4, 20, 21]. Miller *et al.* [9] and Blankenstein *et al.* [22] have reported that LH-RH effects might be manifested on certain breast tumours, not only by inhibition of gonadal hormone secretion but by a direct influence upon cancer cells modulating their functions.

In this paper the direct and indirect effects of LH-RH agonists were investigated on ER-positive and ER-negative human breast cancer cell lines and their xenograft-bearing mice. Our data indicate that ER are maintained at a constant level during the exponential growth phase of MCF-7 cells [23], and that the ER content of MCF-7 xenografts remained constant for up to 6 wk after implantation.

Our study confirmed the presence of specific binding sites for LH-RH in MCF-7 and MDA-MB-231 cell suspensions and membrane fractions of their xenografts in immunosuppressed female mice. The evaluation of these peptide receptors could provide additional characteristics of the biological behaviour of these human breast cancer cells.

The synthetic LH-RH analogue Ovurelin can interfere (25–30%) with the proliferation of MDA-MB-231 human breast cancer cells in culture after a 6-day treatment. Under our experimental conditions, the MCF-7 cell line showed an increase in its growth rate as a response to 10 nM E_2 treatment, while the inhibitory effect of Ovurelin in the MCF-7 cell line was negligible.

In the *in vivo* experiments the treated animals bearing either MCF-7 or MDA-MB-231 xenografts responded to the LH-RH analogues given as Zoladex depot and Decapeptyl depot therapy after 2–3 months of treatment. To stop the tumour growth, a 3–4 wk treatment was required for both Zoladex and Decapeptyl depot therapy.

When treating the animals with 1 μg Ovurelin twice daily, it was surprising that both the ER-positive and ER-negative xenografts of the treated animals increased more rapidly than those of the controls, even after a 1-wk treatment, and the treated tumours became more necrotic than the untreated ones. Ovurelin is not available in depot form, and the dose employed (1 μg/animal/twice daily) did not result in a steady high LH-RH agonist level, so neither direct nor indirect antitumour effect was observed *in vivo*.

On the basis of elevated nER and unchanged cPR levels measured on days 14 and 28 in MCF-7 xenografts, we concluded that the estradiol level in the vicinity of the tumour did not decrease to the castration level. Our results suggest that Ovurelin treatment (1 μg/animal/twice daily) does not desensitize the hypophysis. Contrary to Ovurelin administration, the Decapeptyl depot treatment results in a chemical castration-like effect by the 14th day.

As a result of depot treatment (long-acting, sustained release preparation), the LH-RH agonist exerts an antitumour effect. Since the MDA-MB-231 tumour was found to be ER-negative, it seems possible that the regression of this xenograft results from the direct antitumour action of the LH-RH agonist depot. In case of the ER-positive human breast tumours the mechanism of action of the LH-RH agonists is even more complicated than in the ER-negative ones. The main antitumour effect of the depot LH-RH analogues is exerted through suppression of plasma estradiol levels. In addition, the receptor-mediated direct antitumour effect of the depot LH-RH analogue (which may explain the marked necrosis of the treated tumours) should also be considered.

Both the MCF-7 and MDA-MB-231 human breast tumours were shown to contain LH-RH specific binding proteins. The presence of these receptors might be involved in the control of tumour proliferation by unknown mechanisms. The inhibitory effect of Ovurelin on MDA-MB-231 cells *in vitro* and Zoladex depot and Decapeptyl depot *in vivo*, as well as the stimulating effect of daily Ovurelin treatment *in vivo* on both

xenografts, might be explained by the hypothesis that LH-RH-like peptides have a receptor-mediated regulatory role in breast cancer cells.

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