J. Steroid Biochem. Molec. Biol. Vol. 37, No. 6, pp. 1003–1006, 1990
Printed in Great Britain.

0960-0760/90 $3.00 + 0.00

LHRH-RECEPTORS AND LHRH-AGONIST TREATMENT IN OVARIAN CANCER: AN OVERVIEW

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Summary—Considerable evidence exists that ovarian cancer might be gonadotrophin-dependent. Receptors for LH and FSH have been discovered in these tumors. Proliferation of ovarian cancer cells *in vitro* could be stimulated by gonadotrophins. Withdrawal of LH and FSH in animal models of ovarian cancer inhibited growth of these tumors. Phase-II clinical studies have shown that suppression of endogenous gonadotrophins by LHRH-agonists can be beneficial in women with advanced ovarian cancer. Respective controlled clinical trials are performed at present. Also direct effects of LHRH analogues on ovarian tumors have been reported. An LHRH like protein was found in human ovarian tissue. We discovered a specific LHRH binding site (mol. wt 63.2 kDa) in ovarian cancer tissue which is very similar to other human extrapituitary LHRH binding sites, of the low-affinity, high-capacity type, e.g. in breast cancer and the placenta. In the latter tissues, LHRH or a related substance has been proposed as an autocrine regulator of cellular function. If this was also the case in ovarian cancer, direct effects of LHRH analogs on the tumor cells could be used as additional therapeutical points of attack.

INTRODUCTION

Epithelial ovarian carcinoma is one of the most common causes of cancer death in women. Recently there have been some advances in surgical-, radiation- and cytotoxic chemotherapy of this malignancy. The overall results of these treatments, however, are still disappointing. In addition, modern aggressive chemotherapy is burdened with severe side-effects. One trend in modern oncology has therefore been to reduce at least therapy-induced morbidity, if its efficacy cannot be increased by more and more aggressive regimens. With breast and endometrial cancer this has been partly achieved by the introduction of endocrine treatments, both ablative or additive, taking advantage of the sex-steroid dependence of some of these tumors, which is reflected by the presence of estrogen and/or progestin receptors. Also many ovarian carcinomata contain significant amounts of sex-steroid receptors. Therapeutic approaches, however, using either antioestrogens or progestins have not been satisfactory (for review see [1, 2]).

Epidemiological observations have clearly shown that the number of pregnancies and the duration of the use of oral contraceptives are inversely related to the incidence of ovarian cancer [3–5]. Fathalla [3] suggested that the incessant monthly ovulation, which is characteristical for our species, at least in the twentieth century, is a causal factor for the development of ovarian cancer, due to the repeated rupture of the coelomic epithelium. Another explanation might be that the exposure to relevant gonadotrophin levels in cycling women supports the proliferation of ovarian cancer cells. Another fact supporting the latter theory is that with the advent of the menopause, when gonadotrophin levels rise physiologically, there is a steep increase in the incidence of ovarian carcinomata. If ovarian cancer was LH- or FSH-dependent, suppression of gonadotrophins, most effectively achieved with LHRH-agonists or -antagonists should be beneficial for patients suffering from this disease.

A few years ago, Lamberts and coworkers [6] have shown that in a human ovarian tumor, an arrhenoblastoma, testosterone secretion could be directly inhibited by an LHRH agonist. Also in human breast cancer, direct effects of LHRH

Proceedings of the 2nd International EORTC Symposium on "Hormonal Manipulation of Cancer: Peptides, Growth Factors and New (Anti-)Steroidal Agents", Rotterdam, The Netherlands, 9–11 April 1990.

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analogs have been described. Miller *et al.* [7], for example, showed that *in vitro* the proliferation of certain breast cancer cell lines is inhibited by an LHRH-agonist. These findings are discussed controversially [8] but there seems to be at least agreement that certain breast cancer cells contain binding sites specific for LHRH [9, 10]. The affinity of these binding sites for LHRH or its analogs is lower than that of pituitary receptors. Nevertheless they seem to be able to transmit a message to the cells after binding their ligand. Eidne *et al.* [9] demonstrated that the incubation of breast cancer cells with an LHRH-antagonist dose dependently reduced [^{3}H]thymidine incorporation. This group also identified LHRH and its mRNA in some breast cancer cells [11] and postulated that LHRH is part of an autocrine regulatory mechanism of these tumors.

If a similar system, involving either LHRH- or an LHRH-like substance was present in human ovarian malignoma, another point of attack might exist for the therapeutical use of LHRH-analogs in this disease.

Thus, the application of LHRH-analogs in patients with ovarian cancer could be beneficial via two mechanisms: (1) suppression of LH- and FSH-secretion, thus removing a possible proliferative stimulus; and (2) direct inhibitory effects on the tumor cells, mediated by putative receptors for LHRH or LHRH-like peptides.

Effect of LHRH-agonist-induced suppression of gonadotrophins on the proliferation of ovarian cancer

Apart from the epidemiological data, mentioned above, which suggest a gonadotrophin dependence of ovarian malignoma, several animal and receptor studies have provided data in favor of this theory. Kullander *et al.* [12] and Pour *et al.* [13] prevented the growth of experimental animal tumors by the application of the LHRH-agonist [D-Trp^6]-LHRH. With the same LHRH analog, the growth of the human ovarian carcinoma line OVCAR-3 in nude mice could be inhibited [14]. LH (hCG) and FSH receptors could be demonstrated in some malignant tumors of the human ovary [15, 16]. These findings were contested by other authors [17], but recently at least partly confirmed [18]. The proliferation of ovarian cancer cells *in vitro* was stimulated by hCG and FSH [19, 20].

In 1985, Parmar *et al.* [21] reported on a patient with a relapsed advanced ovarian carcinoma, successfully treated with the LHRH-agonist [D-Trp^6]-LHRH.

Later reports by Kullander *et al.* [12], Parmar *et al.* [22, 23], Jäger *et al.* [24], Kavanagh *et al.* [25] and Bruckner *et al.* [26] have shown that the reduction of LH- and FSH-levels achieved by the administration of LHRH agonists can induce in 20–50% partial remission or lead to stable disease in patients with advanced ovarian cancer relapsing after conventional treatment.

These experimental and clinical observations motivated us to initiate a prospective, placebo-controlled study on the usefulness of LHRH-agonist induced suppression of LH and FSH in patients with ovarian cancer. This multicenter clinical study has been designed to answer the following questions:

1. Is the LHRH-superagonist [D-Trp^6]-LHRH (Decapeptyl) effective in the treatment of ovarian cancer?
2. Can the additional application of Decapeptyl increase the efficacy of the conventional surgical and chemotherapy?
3. Will the additional application of Decapeptyl allow for a less aggressive chemotherapy without a loss of efficacy, thus reducing therapy-induced morbidity?

The study is carried out with patients, in whom an advanced ovarian cancer (FIGO stage III or IV) has been diagnosed for the first time. After surgical treatment and staging, the patients, volunteering for the study (informed consent), are randomized into the Decapeptyl group and the placebo group.

All patients receive a standardized first line polychemotherapy (containing cisplatinum). Decapeptyl or placebo administration are additional and will be continued if a second line chemotherapy will be necessary. Decapeptyl or placebo administration starts within 2 weeks of surgical therapy and is continued for 2 yr or until the death of the patient. A follow-up period of at least 2 yr is planned. At least 100 patients shall be recruited for each, the Decapeptyl and the placebo group. An effect of the Decapeptyl therapy will be accepted, if in the treatment group, survival times will be at least 20% longer than in the control group (for further details see [27]).

Direct effects of LHRH-analogs on human ovarian carcinomata

In the rat, LHRH has been described as a modulator of ovarian function and high-affinity, low-capacity receptors for LHRH have been demonstrated in the ovaries of this species,

comparable to pituitary LHRH receptors (for review see [28, 29]). In the human ovary comparable high-affinity, low-capacity LHRH receptors could not be found [30]. In later studies, however, low-affinity high-capacity LHRH binding sites have been characterized in human corpora lutea [31, 32]. Recently, Aten *et al.* [33] described an LHRH-like protein in human ovaries which might be part of an autocrine regulatory mechanism.

As a first step to elucidate possible direct effects of LHRH-analogs on human ovarian cancer, we checked, whether or not LHRH-binding sites are present in these tumors. Using [^{125}I, D-Ala^{6}-des Gly^{10}]-LHRH ethylamide, we were able to demonstrate low-affinity, high-capacity binding of this LHRH agonist in plasma membranes from a number of human ovarian epithelial carcinomata. The binding was dependent on temperature, time and membrane protein concentration. The analysis of the binding data obtained from inhibition curves with unlabelled LHRH-analogs was consistent with a single class of low-affinity, high-capacity binding sites ($K_a = 1.42 \pm 0.14 \times 10^5$ l/mol; $R = 209 \pm 69 \times 10^{-12}$ mol/mg membrane protein; $n = 32$).

The specificity of the LHRH-binding site was tested using other peptides in concentrations up to 10^{-4} mol/l. Oxytocin, thyrotropin-releasing hormone, and corticotropin-releasing factor did not cause any displacement of [^{125}I]LHRH-A. Somatostatin, however, showed cross-reactivity in some of the tumors tested, whereas it did not displace the LHRH-A in others. Native LHRH, other LHRH agonists and some LHRH antagonists were bound with nearly the same characteristics as the [D-Ala^{6}- des Gly^{10}] LHRH-ethylamide.

Of the 40 ovarian epithelial carcinomata tested, LHRH-agonist binding could be demonstrated in 32 (for futher details see [34]).

Recently, we have been able to solubilize the binding sites from membranes of human ovarian carcinomata retaining their binding activity by using the zwitterionic detergent (3-[3-cholamidopropyl]-dimethylammonio) propanesulfonic acid (CHAPS) [35]. Using a photoaffinity labelling technique and subsequent SDS–polyacrylamide gel electrophoresis we could identify a high molecular weight binding site of 63.2 kDa and a smaller component of 46 kDa [36].

Using the same binding assay, we were able to measure binding sites of the same characteristics in human term placentae, human corpora lutea, human granulosa cells [34] and in both normal and neoplastic human endometrium [37]. Thus in human epithelial carcinomata exists a specific low-affinity, high-capacity binding site for LHRH. It seems to be very similar to LHRH binding sites in human copora lutea, granulosa cells, placental and breast cancer tissue (for further details see [27, 34]). The functional role of this binding site is still obscure. Maybe it is part of an autocrine mechanism as a receptor for LHRH or an LHRH-like peptide formed locally.

Maybe, it is just a phylogenetic residue without any functional role. But even in this case it should be very interesting to correlate the density of the binding site with the histology and the clinical course of the disease to get perhaps another predictive parameter.

There remains a lot of work to be done. At present we are studying, whether or not the proliferation of cancer cells *in vitro* can be modulated by LHRH agonists or antagonists. Preliminary data from our laboratory indicate that the proliferation of the ovarian cancer cell line EFO 27 [20] is dose-dependently inhibited by the LHRH agonist [D-Trp^{6}]-LHRH.

Also the possible activation of LHRH second messenger mechanisms, e.g. the agonist-induced generation of inositol phosphates in ovarian tumor cells is presently under investigation in our laboratory. And of course the demonstration of the formation of LHRH or another natural ligand in ovarian cancer is of crucial importance.

The research on the use of LHRH analogs in ovarian cancer has just started. Thus at the moment only few clinical and experimental data can be presented. But the evidence available today supplies convincing motivation for intensive research in this field. Maybe the result of this work will be that LHRH agonist therapy in ovarian cancer is free of severe side effects but also free of significant effects on tumor growth. But the chance of developing a non-toxic therapy with significant beneficial effects in patients suffering from this fatal disease obliges us to accept this challenge.

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