Gonadotropin-Releasing Hormone Type II Antagonists Induce Apoptotic Cell Death in Human Endometrial and Ovarian Cancer Cells In vitro and In vivo

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Abstract

In human endometrial and ovarian cancers, gonadotropin-releasing hormone type I (GnRH-I), GnRH-II, and their receptors are parts of a negative autocrine/regulatory system of cell proliferation. Based on a tumor-specific signal transduction, GnRH-I and GnRH-II agonists inhibit the mitogenic signal transduction of growth factor receptors and related oncogene products associated with tyrosine kinase activity via activation of a phosphotyrosine phosphatase resulting in down-regulation of cancer cell proliferation. Induction of apoptosis is not involved. In this study, we show that treatment of human endometrial and ovarian cancer cells with GnRH-II antagonists results in apoptotic cell death via dose-dependent activation of caspase-3. The antitumor effects of the GnRH-II antagonists could be confirmed in nude mice. GnRH-II antagonists inhibited the growth of xenotransplants of human endometrial and ovarian cancers in nude mice significantly, without any apparent side effects. Thus, GnRH-II antagonists seem to be suitable drugs for an efficacious and less toxic endocrine therapy for endometrial and ovarian cancers. [Cancer Res 2007;67(4):1750–6]

Introduction

The expression of gonadotropin releasing hormone (GnRH-I) and its receptor as a part of a negative autocrine/paracrine regulatory mechanism of cell proliferation has been shown in a number of malignant tumors, including cancers of endometrium and ovary (1). In these cancers, the in vitro proliferation can be inhibited by agonistic analogues of GnRH-I in a dose- and time-dependent manner (1–5). GnRH-I antagonists also have marked antiproliferative activity in most endometrial and ovarian cancer cell lines tested in vitro, indicating that the dichotomy of GnRH-agonists and antagonists might not apply to the GnRH system in cancer cells (1–5).

GnRH-I agonists have become a cornerstone in the systematic treatment of premenopausal women with estrogen-dependent breast cancer (6). Here, the essential mode of action of GnRH-I agonists is the down-regulation of pituitary gonadotropin secretion, leading to a suppression of ovarian estrogen production, a state called reversible medical castration (7). Single-agent therapy with GnRH-I agonists in doses used for suppression of pituitary gonadotropin secretion has a modest activity in ovariecmetimized patients with ovarian or endometrial cancer, leading, in some cases, to long-lasting remissions or disease stabilization. This effect is probably mediated through the GnRH system in the tumor cells (8). Regarding breast and prostate cancer, the antitumor effects of GnRH-I agonists and antagonists are mainly due to the down-regulation of the hypothalamic-ovarian axis, leading to the suppression of sex steroid production by the gonads. Using the human ovarian cancer cell line OV-1063 xenografted into nude mice, Yano et al. (9, 10) showed a significant inhibition of tumor growth by chronic treatment with the GnRH-I antagonist cetrorelix but not with the GnRH-I agonist triptorelin. As both GnRH-I analogues induced a comparable suppression of the pituitary-gonadal axis, the authors speculated that in vivo antitumor effects of the GnRH-I antagonist cetrorelix were exerted directly on GnRH-I receptors in the tumors.

The classic GnRH-I receptor signal transduction mechanisms, known to operate in the pituitary, are not involved in the mediation of antiproliferative effects of GnRH-I analogues in cancer cells (1). The GnRH-I receptor rather interacts with the mitogenic signal transduction of growth factor receptors and related oncogene products associated with tyrosine kinase activity via activation of a phosphotyrosine phosphatase (PTP) counteracting the epidermal growth factor (EGF)–induced auto-tyrosine phosphorylation of the EGF receptor. Thus, the activity of the extracellular signal-regulated kinases 1 and 2 (ERK1/2) is decreased. Moreover, the EGF-induced expression of the immediate early gene c-fos is reduced. Thus, cell proliferation is decreased (12–14).

Recently, we have shown that GnRH type II (GnRH-II) mediates antiproliferative effects in human endometrial and ovarian cancer cell lines that are significantly greater than those of the GnRH-I agonist triptorelin (11, 15). We could show that the mitogenic effects of growth factors were counteracted by GnRH-II agonist [D-Lys6]GnRH-II, indicating an interaction with the mitogenic signal transduction (16). We have shown that [D-Lys6]GnRH-II reduces EGF-induced auto-tyrosine phosphorylation of EGF receptors via activation of a PTP. EGF-induced activation of ERK1/2 was blocked after treatment with [D-Lys6]GnRH-II. Furthermore, EGF-induced expression of the immediate-early gene c-fos was inhibited (16). These data suggest that the signaling of GnRH-II agonist [D-Lys6]GnRH-II is comparable with that of GnRH-I agonists. Induction of apoptosis was not involved in the antiproliferative effects of GnRH-I or GnRH-II agonists.

In the present study, we have developed and ascertained the effects of antagonists of GnRH-II. These GnRH-II antagonists reduce the growth of endometrial and ovarian cancer cell lines in vitro. In this study, we have assessed whether or not GnRH-II antagonists reduce tumor growth via induction of apoptosis. In addition, we have analyzed the efficacy of the use of GnRH-II antagonists for an antitumor therapy in nude mice.
Materials and Methods

Cell lines and culture conditions. The human endometrial cancer cell lines Ishikawa, Hec-1A, and Hec-1B and the ovarian cancer cell lines EFO-21, OVCAR-3, and SK-OV-3 were obtained from the American Type Culture Collection (Manassas, VA) or the sources detailed previously (2, 3). The Ishikawa, Hec-1A, Hec-1B, EFO-21, and OVCAR-3 cell lines express receptors for GnRH-I and GnRH-II (11, 15–17). The SK-OV-3 cell line is GnRH-I receptor negative but GnRH-II receptor positive (11, 15–17).

The cells were cultured at 37°C in a humidified atmosphere of 5% CO2 in air as previously described (2–4).


Apoptosis assays. Cells grown in six-well plates were treated without or with GnRH-II antagonists in a final concentration of 10−8 mol/L or with increasing concentrations (10−9 to 10−6 mol/L) of the GnRH-II antagonists for 72 or 96 h before they were harvested. To quantify apoptosis, we used a procedure similar to that described by Nicoletti et al. (18) that was based on detecting advanced DNA degradation. Briefly, a pellet containing 1 × 106 cells was gently resuspended in 500 μl of hypotonic fluorochrome solution containing 0.1% Triton X-100 (Sigma, Deisenhofen, Germany), 0.1% sodium citrate, and 50 mg/mL propidium iodide (Sigma). The cell suspensions were placed at 4°C in the dark overnight before flow cytometry analysis of cellular DNA content on a FACSCalibur equipment (Becton Dickinson Immunocytometry Systems, Mountain View, CA) was done with CellQuest software (Becton Dickinson Immunocytometry Systems).

Mitochondrial membrane potential. For determination of GnRH-II antagonist–induced loss of mitochondrial membrane potential, the cells were treated without or with increasing concentrations (10−9 to 10−6 mol/L) of the GnRH-II antagonists for 72 h. After incubation, the cells were washed with PBS once, and the mitochondrial membrane potential was detected using the JC-1 mitochondrial membrane potential detection kit according the instructions of the manufacturer (Biotium, Hayward, CA).

Western blot analysis of caspase-3 activity. For determination of GnRH-II antagonist–induced caspase-3 activity, the cells were treated without or with increasing concentrations (10−9 to 10−6 mol/L) of the GnRH-II antagonists for 72 h. After incubation, the cells were detached immediately with 0.5 g trypsin (Biochrom, Berlin, Germany) and 5 mmol EDTA in 1 liter PBS/bovine serum albumin. The pellets were washed twice with PBS and resuspended with CellLytic buffer (Sigma) containing protease inhibitors (Sigma). Equal amounts of protein per sample were used and diluted to equal volumes with Laemmli buffer (19). The cell lysates were separated on SDS-PAGE (15%, ProSieve 50Gel Solution, Cambrex, Verviers, Belgium) under reducing conditions and transferred to nitrocellulose membranes (HybonD-ECL, GE Healthcare Europe, Munich, Germany). The bands were analyzed using the peroxidase substrate, hydrogen peroxide, and the stable chromogen diaminobenzidine. Counterstaining was done using Meyer's hematoxylin for 10 s. The slides were then dehydrated, cleared, mounted with Permount, and studied by light microscopy.

Statistical analysis. All experiments were repeated three times with different passages of the respective cell lines. Data were tested for significant differences using Mann-Whitney U test. The data from the dose response experiments were tested for significant differences by one-way ANOVA followed by Student-Newman-Keuls’ test for comparison of individual groups, after a Bartlett test had shown that variances were homogenous.

Results

Effects of GnRH-II Antagonists on Induction of Apoptosis In vitro

After 72 h of exposure of EFO-21 human ovarian cancer cells to cytotoxic agent doxorubicin at 10−7 mol/L (Fig. 1A, middle; positive control) or GnRH-II antagonist [Ac-D2Nal1, D-4Cpa2, D-3Pal3,6, Leu8, D-Ala10]GnRH-II (Fig. 1A, right) at 10−7 mol/L, cells showed morphologic changes characteristic of apoptosis, including membrane blebbing, detachment, and nuclear and cytoplasmic condensation. Without treatment (Fig. 1A, left; negative control), no morphologic signs of programmed cell death were observed. Flow cytometry analysis revealed advanced DNA fragmentation, based on which the percentage of apoptotic cells was determined (Fig. 1B and C).

Treatment with GnRH-II antagonists in a final concentration of 10−5 mol/L. In SK-OV-3 ovarian cancer cells, treatment with 10−5 mol/L of the GnRH-II antagonists [Ac-D2Nal1, D-4Cpa2].
The effects of the GnRH-II antagonists on induction of apoptotic cell death could be confirmed by the loss of the mitochondrial membrane potential (ΔΨ). Treatment of OVCAR-3 ovarian cancer cells with cytotoxic agent doxorubicin (positive control) or with increasing concentrations (10⁻¹¹ to 10⁻⁵ mol/L) of the GnRH-II antagonist [Ac-D2Nal¹, D-4Cpa², D-3Pal³, D-Lys⁶, Leu⁹, D-Ala¹⁰]GnRH-II for 72 h resulted in a reduction of mitochondrial membrane potential (Fig. 2A). After treatment with 10⁻⁵ mol/L of the cytotoxic agent doxorubicin (positive control), caspase-3 activity was decreased to 82.46 ± 3.37% of control (= 100%; not significant). Treatment with 10⁻⁵ mol/L of the GnRH-II antagonist resulted in a decrease of mitochondrial membrane potential to 86.40 ± 5.98% of control (= 100%; not significant). Treatment with 10⁻⁵ mol/L of the GnRH-II antagonist resulted in a decrease of mitochondrial membrane potential to 86.40 ± 5.98% of control (= 100%; not significant).

**Dose-response experiments.** In Ishikawa endometrial cancer cells, treatment with increasing concentrations (10⁻⁹ to 10⁻⁵ mol/L) of the GnRH-II antagonist [Ac-D2Nal¹, D-4Cpa², D-3Pal³, D-Ala¹⁰]GnRH-II resulted in increased amounts of apoptotic cells (Fig. 1C). After treatment with 10⁻⁹ mol/L of the GnRH-II antagonist, 31.13 ± 2.48% of apoptotic cells were counted (not significant versus control). Treatment with 10⁻⁷ mol/L of the GnRH-II antagonist resulted in 33.85 ± 4.02% of apoptotic cells (not significant versus control). The effects were maximal at 10⁻⁷ mol/L concentrations of the GnRH-II antagonist and corresponded to 56.58 ± 18.33% of apoptotic cells (P < 0.01 versus control).

Experiments using the ovarian cancer cell lines SK-OV-3, EFO-21, or OVCAR-3 or the endometrial cancer cell lines Ishikawa or Hec-1A gave comparable results (data not shown).

**Effects of GnRH-II Antagonists on Mitochondrial Membrane Potential**

The effects of the GnRH-II antagonists on induction of apoptotic cell death could be confirmed by the loss of the mitochondrial membrane potential (ΔΨ). Treatment of OVCAR-3 ovarian cancer cells with cytotoxic agent doxorubicin (positive control) or with increasing concentrations (10⁻¹¹ to 10⁻⁵ mol/L) of the GnRH-II antagonist [Ac-D2Nal¹, D-4Cpa², D-3Pal³, D-Lys⁶, Leu⁹, D-Ala¹⁰]GnRH-II for 72 h resulted in a reduction of mitochondrial membrane potential (Fig. 2A). After treatment with 10⁻⁵ mol/L of the cytotoxic agent doxorubicin (positive control), caspase-3 activity was decreased to 82.46 ± 3.37% of control (= 100%; not significant). Treatment with 10⁻⁵ mol/L of the GnRH-II antagonist resulted in a decrease of mitochondrial membrane potential to 86.40 ± 5.98% of control (= 100%; not significant). Treatment with 10⁻⁵ mol/L of the GnRH-II antagonist resulted in a decrease of mitochondrial membrane potential to 86.40 ± 5.98% of control (= 100%; not significant). Treatment with 10⁻⁵ mol/L of the GnRH-II antagonist resulted in a decrease of mitochondrial membrane potential to 86.40 ± 5.98% of control (= 100%; not significant). Treatment with 10⁻⁵ mol/L of the GnRH-II antagonist resulted in a decrease of mitochondrial membrane potential to 86.40 ± 5.98% of control (= 100%; not significant).
Concentrations (10⁻¹⁰ to 10⁻⁶ mol/L) of the GnRH-II antagonist resulted in an increase of caspase-3 activity to 192.55 ± 8.05% of control (P < 0.001). After treatment with 10⁻⁷ mol/L of the GnRH-II antagonist, caspase-3 activity was increased to 264.17 ± 15.16% of control (P < 0.001). The effects were maximal at 10⁻⁶ mol/L concentrations of the GnRH-II antagonist and corresponded to an increase of caspase-3 activity to 369.54 ± 19.87% of control (P < 0.001).

Experiments using the ovarian cancer cell lines SK-OV-3 or EFO-21 or the endometrial cancer cell lines Ishikawa or Hec-1A gave comparable results (data not shown).

**Effects of Pertussis Toxin on GnRH-II Antagonist Action**

To show the linkage between GnRH-II antagonist–induced activation of G-protein Goi and activation of caspase-3, the effect of pertussis toxin on GnRH-II antagonist–induced caspase-3 activity was analyzed (Fig. 2C). After treatment with 10⁻⁷ mol/L of the GnRH-II antagonist, caspase-3 activity was increased to 198.7 ± 56.7% of control (P = 100%; not significant). The GnRH-II antagonist–induced increase of caspase-3 activity was reduced by pertussis toxin pretreatment (20 ng/mL) to 160.9 ± 41.3% of control (P = 100%; not significant).

**Effects of GnRH-II Antagonists on Tumor Growth**

**In vivo**

To show the proof-of-principle of an antitumor therapy using GnRH-II antagonists, nude mice bearing xenografted human endometrial or ovarian tumors s.c. were treated with GnRH-II antagonists.

**Ovarian cancer.** Female nude mice bearing OVCAR-3 ovarian tumors s.c. were treated without (control) or with 25 nmol per injection of GnRH-II antagonists [Ac-D2Nal₁, D-4Cpa₂, D-3Pal₃, D-Lys₆, D-Ala¹⁰]GnRH-II (AG-1), [Ac-D2Nal₁, D-4Cpa₂, D-3Pal₃, D-Lys₆, Leu⁶, D-Ala¹⁰]GnRH-II (AG-2), or [Ac-D2Nal₁, D-4Cpa₂, D-3Pal₃, Leu⁶, D-Ala¹⁰]GnRH-II (AG-3). The treatments were repeated every day, and tumor volumes were measured every 2 days. The mice were killed after 10 days of treatment. The increase of the tumor volume of the mice receiving therapy with GnRH-II antagonists was lower than with the control animals. After 4 days of treatment, the differences became significant (P < 0.01). Due to high variances within the control group, the differences were not significant anymore after 10 days of treatment. Side effects were not observed.

**Induction of apoptosis in vivo.** To show that GnRH-II antagonists induce apoptosis within the tumor xenotransplants, we did TUNEL assays (Fig. 3B and C). In the TUNEL assay, the enzyme terminal deoxynucleotidyl transferase labels 3'-OH DNA ends, generated during apoptosis, with biotinylated nucleotides. The latter are detected by immunoperoxidase staining. The amount of apoptotic cells in tumors of nude mice receiving therapy with GnRH-II antagonists was higher than with the control animals (Fig. 3B).

**Endometrial cancer.** Female nude mice bearing Hec-1B endometrial tumors s.c. were treated without (control) or with 25 nmol per injection of GnRH-I agonist triptorelin (control II) or with 25 nmol per injection of the GnRH-II antagonists [Ac-D2Nal₁, D-4Cpa₂, D-3Pal₃, D-Lys₆, D-Ala¹⁰]GnRH-II (AG-1), [Ac-D2Nal₁, D-4Cpa₂, D-3Pal₃, D-Lys₆, Leu⁶, D-Ala¹⁰]GnRH-II (AG-2), or [Ac-D2Nal₁, D-4Cpa₂, D-3Pal₃, Leu⁶, D-Ala¹⁰]GnRH-II (AG-3). The treatments were repeated every 2 days, and tumor volumes were measured one time per week. The mice were killed after 21 days of treatment. The increase of the tumor volume of the mice receiving therapy with GnRH-II antagonists was lower than with the control animals. After 7 days of treatment, the differences became highly significant (P < 0.001) and remained highly significant (Fig. 4B; P < 0.001). Side effects were not observed.
Points, was repeated everyday. All experimental groups consisted of five animals.

Treatment (AG1), [Ac-D2Nal1,D-4Cpa2,D-3Pal3,D-Lys6,Leu8,D-Ala10]GnRH-II (AG2), and [Ac-D2Nal1,D-4Cpa2,D-3Pal3,6,Leu8,D-Ala10]GnRH-II (AG3). Treatment was repeated every day. All experimental groups consisted of five animals.

Figure 3. A, tumor volume of OVCAR-3 human ovarian cancers xenografted into nude mice. The mice were treated without (control) or with 25 nmol of GnRH-II antagonists [Ac-D2Nal1,D-4Cpa2,D-3Pal3,6,Leu8,D-Ala10]GnRH-II (AG1), [Ac-D2Nal1,D-4Cpa2,D-3Pal3,D-Lys6,Leu8,D-Ala10]GnRH-II (AG2), and [Ac-D2Nal1,D-4Cpa2,D-3Pal3,6,Leu8,D-Ala10]GnRH-II (AG3). Treatment was repeated every day. All experimental groups consisted of five animals.

Discussion

Previous work showed direct antiproliferative effects of GnRH-I agonists and antagonists in GnRH receptor–positive human endometrial and ovarian cancer cells (1–5). GnRH receptor signal transduction mechanisms that operate in the pituitary gonadotrophs are not involved in the antiproliferative effects of GnRH-I analogues in these cancer cells (1). GnRH receptors in the pituitary couple to G protein q and activate phospholipase C and protein kinase C (20), whereas GnRH receptors in reproductive tumors couple to pertussis toxin–sensitive G proteins of the Gi family and activate phosphotyrosine phosphatase (21, 22). The difference in the signal transduction pathway linked to the GnRH receptor between the pituitary and peripheral tumors may be one explanation for the antagonistic action of GnRH-I antagonist in the pituitary and the agonistic action of GnRH-I antagonist in cancer cells. However, the dichotomy of GnRH-I agonists and antagonists does not exist in tumor cells because the proliferation of cancer cell lines expressing GnRH receptors is inhibited by both agonistic and antagonistic analogues of GnRH-I (12). Another explanation could be that the agonistic effects of GnRH-I antagonist cetrorelix are due to a cross-reaction with an additional GnRH receptor. We have recently shown that in cell lines affected by both GnRH-I agonist triptorelin and GnRH-II antagonist cetrorelix, the effects of the former were abrogated after GnRH-I receptor knockout, whereas the effects of cetrorelix persisted (11). These findings suggested that the antiproliferative/agonistic effects of the GnRH-I antagonist cetrorelix are not mediated through the GnRH-I receptor.

Recently, we have shown that GnRH-II mediates antiproliferative effects in endometrial and ovarian cancer cells that are significantly greater than those of the GnRH-I agonist triptorelin (15). The antiproliferative effects of the GnRH-II agonist triptorelin and the GnRH-II agonist [D-Lys6]GnRH-II in these cell lines were not due to increased apoptosis (15, 23, 24). In our system, triptorelin and [D-Lys6]GnRH-II interfered with EGF-induced signal transduction observed. To exclude that the antitumor effects of the GnRH-II antagonists are due to an interaction with the pituitary GnRH receptors, a second control group (control II) was treated with 25 nmol per injection of the GnRH-I agonist triptorelin. The increase of the tumor volume of the mice receiving therapy with GnRH-I agonist triptorelin was nearly the same than with the animals of control I. No antitumor effects using triptorelin were observed.

GnRH-I and GnRH-II receptor density after treatment with GnRH-II antagonists in vivo. After the end of the experiments, GnRH-I receptor and GnRH-II receptor expression was determined. In OVCAR-3 ovarian cancers and in HEC-1B endometrial cancers, the density of GnRH-I and GnRH-II receptors after treatment with GnRH-II antagonists was the same, compared with the control tumors (data not shown).

Figure 4. A, tumor volume of Hec-1B human endometrial cancers xenografted into nude mice. Treatment without (control) 1) or with GnRH-I agonist triptorelin (control 2) or with 25 nmol of GnRH-II antagonists [Ac-D2Nal1,D-4Cpa2,D-3Pal3,6,Leu8,D-Ala10]GnRH-II (AG1), [Ac-D2Nal1,D-4Cpa2,D-3Pal3,D-Lys6,Leu8,D-Ala10]GnRH-II (AG2), and [Ac-D2Nal1,D-4Cpa2,D-3Pal3,6,Leu8,D-Ala10]GnRH-II (AG3). Treatment was repeated every day. All experimental groups consisted of five animals. Points, mean; bars, SE.

B, TUNEL assay showing apoptotic cells in OVCAR-3 human ovarian tumors grown in nude mice without (B) or with (C) therapy with GnRH-II antagonist [Ac-D2Nal1,D-4Cpa2,D-3Pal3,6,Leu8,D-Ala10]GnRH-II. Columns, mean of data obtained from five animals; bars, SE. a, P < 0.01, versus control.
and completely inhibited EGF-induced autophosphorylation of EGF receptor and induction of mitogen-activated protein kinase (ERK1/2) activity (1, 16). This is different with GnRH-II antagonists. In this study, we have shown that treatment of human endometrial and ovarian cancer cells with GnRH-II antagonists resulted in apoptotic cell death. The fact that treatment with GnRH-II antagonists resulted in a loss of mitochondrial membrane potential and an increase of caspase-3 activity in cultured endometrial and ovarian cancer cells suggests that GnRH-II antagonists induce apoptosis in these cells at least in part through activation of the intrinsic apoptotic pathway. Recently, Maiti et al. reported that high concentrations of the GnRH-II antagonist triptorelin-I but not GnRH-I antagonist cetorelix induced prostate cancer cell death in vitro, probably through an apoptotic process (25).

In earlier studies, we have shown that a functional GnRH-II receptor may exist in human cancers (11, 15–17, 24). GnRH-II agonist [D-Lys6]GnRH-II had strong antiproliferative effects in the GnRH-I receptor–negative human ovarian cancer cell line SKOV-3 (15). After knockout of GnRH-I receptor expression, the antiproliferative effects of GnRH-I agonist triptorelin on originally GnRH-I receptor–positive endometrial and ovarian cancer cell lines were abrogated, whereas the growth inhibitory effects of [D-
Lys6]GnRH-II were still the same as observed in nontransfected cells (11). Because treatment of the GnRH-I receptor–negative but GnRH-II receptor–positive ovarian cancer cell line SK-OV-3 with GnRH-II antagonists resulted in induction of apoptosis, GnRH-II antagonists may be effective via the GnRH-II receptor. However, we cannot exclude that in addition GnRH-II antagonists induce apoptosis via the GnRH-I receptor. Further experiments (i.e., GnRH-I receptor and GnRH-II receptor knockout experiments) are required to analyze the cross-reactivity of GnRH-II antagonists.

The antiproliferative actions of GnRH-I and GnRH-II analogues were predominantly mediated through the pertussis toxin–sensitive G protein αi (13, 16). To show the linkage between GnRH-II antagonist-induced GnRH-II receptor activation and induction of apoptosis, the effects of pertussis toxin on GnRH-II antagonist–induced caspase-3 activity were analyzed. The GnRH-II antagonist–induced increase of caspase-3 activity was reduced by pertussis toxin, indicating that it is mediated at least in part by G protein αi. Comparable data were obtained by Imai et al. (26). They have shown that GnRH-I antagonist cetorelix–induced apoptotic cell death of human ovarian cancer cells was mediated through pertussis toxin–sensitive Gi protein–linked GnRH receptor.

We could show the proof-of-principle of an antitumor therapy using GnRH-II antagonists in vivo in nude mice bearing s.c. xenografts of human endometrial or ovarian tumors. Nude mice bearing Hec-1B human endometrial tumors or OVCAR-3 human ovarian tumors were treated with the GnRH-II antagonists [Ac-D2Nal1, D-4Cpa2, D-3Pal3, D-Lys6, D-Ala10]GnRH-II, [Ac-D2Nal1, D-4Cpa2, D-3Pal3, D-Lys6, Leu8, D-Ala10]GnRH-II, or [Ac-D2Nal1, D-4Cpa2, D-3Pal3, D-Ala10]GnRH-II. The increase of the tumor volume of the mice receiving therapy with GnRH-II antagonists was significantly lower than with the control animals. Toxic side effects were not observed.

To exclude that the antitumor effects of the GnRH-II antagonists are mainly due to an interaction with the pituitary GnRH receptors and a subsequent reduction of ovarian estrogen production, a control group was treated with GnRH-I agonist triptorelin. Because no antitumor effects using triptorelin were observed, down-regulation of the hypothalamic-ovarian axis cannot be the main reason for the antitumor effects of GnRH-II antagonists. GnRH-II antagonists seem to affect the tumor cells directly by inducing apoptosis. The amount of apoptotic cells, counted using TUNEL assay, in tumors of nude mice receiving therapy with GnRH-II antagonists was higher than with the control animals. Therefore, the antitumor effects of the GnRH-II antagonists in vivo are due to induction of apoptosis. However, it is not excluded that the down-regulation of the hypothalamic-ovarian axis may have a little part on antitumor effects of GnRH-II antagonists. Considering that, after 21 days of treatment with GnRH-II antagonists, no down-regulation of GnRH-I receptor and GnRH-II receptor expression was found in the tumors that were grown in vivo, GnRH-II antagonists seem to be suitable for chronic or repeated therapy.

GnRH-II antagonists seem to be suitable drugs for an efficacious and less toxic targeted therapy for endometrial and ovarian cancers. Our findings could be the basis for a further evaluation in clinical trials. GnRH-I agonists and GnRH-I antagonists have been widely used in the therapy of cancer (8, 27–30) and endometriosis (31–33) as well as in reproductive medicine (34–38). Their effects are mainly due to the down-regulation of the hypothalamic-ovarian axis. Because we could not observe antitumor effects in vivo after down-regulation of the hypothalamic-ovarian axis using the GnRH-I agonist triptorelin, the down-regulation of the hypothalamic-ovarian axis alone cannot be responsible for the antitumor effects of GnRH-II antagonists.

In conclusion, we have shown that GnRH-II antagonists exert antitumor effects in human endometrial and ovarian cancer cells by inducing apoptosis through the caspase cascade. The proof-of-principle of an antitumor therapy using GnRH-II antagonists could be shown in vivo in nude mice bearing human endometrial or ovarian tumors s.c. As toxic side effects were not observed, this approach should be further studied.

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References

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Announcements

MEETING OF THE RADIATION RESEARCH SOCIETY

The annual meeting of the Radiation Research Society will be held at the State University of Iowa, Iowa City, on June 22-24, 1953. The Society will be the host of the University, and all meetings will be held on the campus. The program will consist of: (1) Two symposia, one on "The Effects of Radiation on Aqueous Solutions," which includes the following speakers: E. S. G. Barron, Edwin J. Hart, Warren Garrison, J. L. Magee, and A. O. Allen. The second is "Physical Measurements for Radiobiology" and companion talks by Ugo Fano, Burton J. Moyer, G. Failla, L. D. Marinelli, and Payne S. Harris. (2) On Monday night, June 22, a lecture by Dr. L. W. Alvarez on meson physics has been tentatively scheduled. On Tuesday night, June 23, Dr. L. H. Gray of the Hammersmith Hospital, London, will speak on a topic to be announced. Dr. Gray's lecture is sponsored by the Iowa Branch of the American Cancer Society. Those desiring to report original research in radiation effects, or interested in attending or desiring additional information, please contact the Secretary of the Society, Dr. A. Edelmann, Biology Department, Brookhaven National Laboratory, Upton, L.I., New York.

ERRATUM

The following correction should be made in the article by Beck and Valentine, "The Aerobic Carbohydrate Metabolism of Leukocytes in Health and Leukemia. I. Glycolysis and Respiration," November, 1952, page 891; substitute for the last paragraph:

The data in Table 8 permit several interesting calculations. If one compares the amount of glucose actually disappearing with the sum of the amount equivalent to lactic acid produced plus that equivalent to O₂ consumption, it is seen that the amount of glucose "cleavage products" exceeds the amount of glucose utilized by 12 per cent in N and 27 per cent in CML and is exceeded by the glucose utilized by 16 per cent in CLL. If the assumption is made that, in this respect, the myeloid and lymphoid cells of leukemia are similar to those of normal blood, it may be that the computed normal figure represents a summation of the myeloid (M) and lymphoid (L) cells that make up the normal leukocyte population. Thus, if  M = +0.27 and  L = -0.16 and the normal differential is 65 per cent M and 35 per cent L, then

\[ 0.65 (+0.27) + 0.35 (-0.16) = +0.12 \]

a figure identical to the observed +0.12 for normal leukocytes.
Gonadotropin-Releasing Hormone Type II Antagonists Induce Apoptotic Cell Death in Human Endometrial and Ovarian Cancer Cells In vitro and In vivo

Stefanie Fister, Andreas R. Günthert, Günter Emons, et al.


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