

Effect of GnRH analogues and octreotide treatment on apoptosis and the cell proliferation of endometrium adenocarcinoma cell lines

Endometriyal adenokanser hücre serilerinde GnRH analogları ve oktreotidin apoptozis ve hücre proliferasyonu üzerindeki etkileri

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Abstract

Objective: The aim of this study was to compare apoptotic and antiproliferative effects of gonadotropin-releasing hormone analogues and their combination with octreotide on endometrioid endometrial cancer cell lines.

Material and Method: Women diagnosed with endometrioid adenocarcinoma at the department of Gynecology and Obstetric of Kocaeli University Medical School were included in this research. Endometrium cancer cell lines obtained from three patients were used for this study. After trypsinization in 0.5% in calcium magnesium, free phosphate buffer solution (CMFPBS) cells were seeded on glass slides in 24-well plates containing DMEM-F12 medium and 10% fetal calf serum as culture medium. Cells were incubated for 24 hours at 37°C in 5% CO₂. GnRH agonist leuprolide (Lucrin 1 µmol/L), GnRH antagonist ganirelix (Orgalutran 1 µmol/L), leuprolide with octreotide (Sandostatin 10-6 mol/L), ganirelix with octreotide and no drug were added to the wells. Apoptosis and cells proliferations were evaluated after 12, 24, 48 and 72th hours of incubation. The percentage of apoptotic cells was evaluated by TdT mediated biotin-dUTP nick-end labeling (TUNEL) method; cell proliferation was assessed by bromodeoxyuridine (BrdU) incorporation.

Results: Apoptotic index in grade I EEC cell line among ganirelix-octreotide treated cells and leuprolide-octreotide combination therapy were respectively higher than the untreated control ($p < 0.001$, $p = 0.001$). The number of apoptotic cells in grade II EEC cell line among leuprolide-octreotide and leuprolide were significantly ($p < 0.001$, $p < 0.001$) higher than in controls. In grade III EEC cell line, the number of TUNEL positive cells among leuprolide, ganirelix and ganirelix-octreotide therapy groups were significantly higher than in untreated control. Time dependent antiproliferative effect was obtained with leuprolide and leuprolide-octreotide in grade I EEC ($p < 0.001$, $p < 0.001$). Grade II EEC cell line is not influenced by hormone therapies. However, the antiproliferative effect was obtained with ganirelix, leuprolide and leuprolide-octreotide in grade III cell line.

Conclusion: GnRH analogues appears to have a direct effect, enhancing the apoptotic index and decreasing the cell proliferation in endometrial adenocarcinoma cell lines.

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Key words: Endometrial cancer, gonadotropin-releasing hormone analogues, octreotide, apoptosis, cell proliferation

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Özet

Amaç: Bu çalışmanın amacı endometriyal adenokanser hücre serilerinde GnRH analogları ve oktreotidin apoptozis ve hücre proliferasyonu üzerindeki etkilerini karşılaştırmaktır.

Gereç ve Yöntemler: Kocaeli Üniversitesi Tıp Fakültesi Kadın hastalıkları ve Doğum bölümünde tanı almış olan endometriyal adenokanser vakaları çalışmaya alındı. Üç hastadan alınmış olan endometriyal kanser hücre serileri bu çalışmada kullanıldı. Tripinizasyon için %0.5 lik kalsiyum magnezyum, free phosphate buffer solusyonunda (CMFPBS) bekletilen hücreler daha sonra DMEM-F12 mediyumu ve %10 dana fetus serumu içeren dishlere ekildi. Hücreler 37°C in %5 CO₂ de 24 saat inkübe edildiler. GnRH agonist leuprolide (Lucrin 1 µmol/L), GnRH antagonist ganirelix (Orgalutran 1 µmol/L), leuprolide ve oktreotide (Sandostatin 10-6 mol/L), ganirelix ve oktreotide ve ilaçsız gruplar oluşturuldu. Apoptozis ve hücre proliferasyonu inkübasyondan sonraki 12, 24, 48 ve 72. saatlerde değerlendirildi. Apoptotik hücre oranı TUNEL yöntemi ile, hücre proliferasyonu ise bromodeoxyuridine (BrdU) ilavesiyle değerlendirildi.

Bulgular: Grade I endometriyal kanser hücre serisindeki apoptotik index ganirelixoktreotide ve leuprolide-oktreotide combination tedavileri alan gruplarda tedavisiz gruba göre anlamlı olarak daha yüksek olarak saptandı ($p < 0.001$, $p = 0.001$). Grade II endometriyal kanser hücre serisindeki apoptotik index sayısı leuprolide-oktreotide ve leuprolide gruplarında kontrol gruba göre anlamlı olarak daha yüksek olarak saptandı ($p < 0.001$, $p < 0.001$). Grade III endometriyal kanser hücre serisindeki TUNEL pozitif hücre oranı leuprolide, ganirelix ve ganirelixoktreotide tedavisi alan gruplarda kontrol grubuna göre anlamlı olarak daha yüksek idi. Zamandan bağımsız antiproliferatif etkinlik leuprolide ve leuprolide-oktreotide gruplarında grade I hücre serilerinde gözlemlendi ($p < 0.001$, $p < 0.001$). Grade 2 Grade I endometriyal kanser hücre serisinin hormon tedavisinden etkilenmediği görüldü. Buna karşın ganirelix, leuprolide ve leuprolide-oktreotide gruplarında Grade III endometriyal kanser hücre serisinde antiproliferatif etkinlik saptandı.

Sonuç: Öyle görülmektedir ki, endometriyal adenokanser hücre serilerinde GnRH analoglarının apoptotik indeksi yükseltme ve hücre proliferasyonu azaltma mekanizmaları ile direkt etkisi vardır.

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Anahtar kelimeler: Endometriyal kanser, gonadotropin-releasing hormone analogları, oktreotide, apoptozis, hücre proliferasyonu

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Introduction

Endometrial carcinoma is the most common malignancy of the female genital tract (1). Despite the dominance of early stage disease, surgical treatment and/or irradiation are not curative for advanced endometrial cancer and the number of reported cancer deaths is increasing. Chronic elevated level of estrogen without the inhibitory effects of progesterone are considered stimuli for abnormal endometrial cell proliferation. Also, mutations in the tumour suppressor genes and microsatellite instability (MI) are common genetic abnormalities in endometrioid endometrial cancers (EEC), and distinguish these lesions from other histological subtypes of endometrial carcinomas (2).

Apoptosis and proliferation are the main factors in controlling both benign and malignant cell populations. Apoptosis is a physiological process leading to cell death characterized by cell shrinkage, membrane blebbing and DNA fragmentation and it is an important phenomenon existing in normal endometrium, regulated by sex steroids during the menstrual cycle (3, 4). In conditions where the concentrations of sex steroids are altered, the expression of apoptosis related proteins is susceptible to change. If the balance of the regulation of cell cycle is affected it thus induces a potential loss in the control of cell survival and may progress to cancer progression.

As endometrial cancer develops from generally hormone dependent cells, endocrine treatment has been the traditional palliative therapy of advanced or recurrent disease. Progestins have been used and currently GnRH analogues and their mechanism of effect have been investigated by many researchers. It has been demonstrated that about 50% to 80% of endometrial cancer express GnRH receptors, and recent researches have been focused on the possible use of GnRH agonists and antagonists as a potential target for the treatment of endometrial cancer (5-7).

Octreotide acetate is a synthetic octapeptide analogue of naturally occurring somatostatin with similar pharmacological effects, but with a prolonged duration of action. It may act directly and specifically on neoplastic cells or indirectly via peptides and/or other substances that are crucial for neoplastic growth.

The aim of the study is to compare the apoptotic and proliferative effect of leuprolide, ganirelix, leuprolide combined with octreotide, ganirelix combined with octreotide and untreated control in human endometrioid adenocarcinoma cell culture to evaluate possible clinical use of these hormones for future treatment of advanced/ recurrent endometrial carcinoma.

Material and Methods

Tissue samples

Surgical specimens were obtained from three patients undergoing hysterectomy for endometrial cancer at the Department of Gynecology and Obstetrics of the Kocaeli University Medical School. All patients were post-menopausal, aged 56, 65 and 71 years. Tumor specimens were placed in ice cold phosphate buffer solution (PBS) immediately after surgical removal and representative portions were excised to prepare the materials for histological frozen section. Approximately 1 mm³ tumor tissue were utilised for the cell culture and the residue was sent for histological examination. Tumor grading of the paraffin-

embedded tissue blocks were compatible with the preoperative diagnosis. This investigation was approved by the Institutional Ethics Committee of the Kocaeli University, School of Medicine. Informed written consent was obtained from all subjects.

Cell culture

Samples of human endometrioid type adenocarcinoma were obtained under sterile conditions in the surgical pathology unit. The tissue was immediately placed into the culture medium and processed within 60 minutes of collection. Single cells obtained by mechanical disruption were separated from the clumps by sedimentation and then removed. The bigger clumps that sedimented were digested in a 37°C shaking water-bath for 1 hour with 1mg/ml collagenase B ve 0.1 mg/ml DNAase. The tissue was then washed in PBS.

After removal of the supernatant, pellets which had been diluted with one ml of the washing medium were filtrated through sieve number 46 (cell-dissociation sieve, Sigma-Aldrich) and the ultrafiltrate was seeded on 25 cm² tissue culture flasks and embedded 10 ml DMEM-F12 medium with 10% fetal calf serum. The cells were cultured in a humidified atmosphere of 5% CO₂ and 95% air at 37°C and passaged every 3 days after the 5th day of incubation. After trypsinization in 0.5% in calcium magnesium free phosphate buffer solution (CMF-PBS), the cells were seeded on glass slides in 24-well plates containing DMEM-F12 (Dulbecco's modified Eagle's minimal essential medium) medium and 10% fetal calf serum as culture medium.

Incubation

Cells were incubated for 24 hours at 37° C in 5% CO₂. Leuprolide acetate (Lucrin®, Abbott, Chicago MI, USA) as GnRH agonist and ganirelix (Orgalutran®, Organon) as GnRH antagonist in concentrations of 1 µmol/L; and a combination of leuprolide with octreotide 10⁻⁶ mol/L (Sandostatin®, Novartis, Quebec, Canada) and ganirelix with octreotide as somatostatin analogue were added to the wells. After 12 hours cells were removed for analysis of apoptosis and after 24, 48, and 72 hours groups, media were removed 1 hr before the end points of each interval and the cells were incubated in 1 ml of medium containing 20µM BrdU for the last hour.

Measurement of apoptosis

The percentage of apoptotic cells was assessed by the TUNEL technique following the manufacturer's instructions (In situ cell death detection kit, POD, Cat No.1 684 817, Roche Diagnostic) in the endometrial cancer cells cultures 12 hours after the addition of GnRH agonist Lucrin 1 µmol/L (Leuprolide®, Abbott, Chicago MI, USA), GnRH antagonist Orgalutran 1 µmol/L (Ganirelix®, Organon) and their combination with somatostatin analogue octreotide 10⁻⁶ mol/L (Sandostatin®, Novartis). The cells were fixed for 30 min in 4% paraformaldehyde at room temperature. The cells were permeabilized with 0.1% TritonX-100 and 0.1% sodium citrate for 2 min at 4°C and were then incubated at 37°C for 60 min in the dark in 50 µL TUNEL reaction mixture. Thereafter they were incubated with 50 µL converter at room temperature for 20 min.

BrdU incorporation in vitro

Detections of BrdU-labeled cells was performed using standard avidin-biotin complex methods immunoperoxidase kits

(LabVision) with primary BrdU antibody (NeoMarkers). The cells were fixed in methanol at -18°C for 1-2 min, allowed to air dry, then stored at -20°C until all coverslips were ready for processing. Cells were rehydrated in the PBS for 5 min, followed by immersion in 2N HCl for 1 hr at room temperature. The cells were incubated in 0.1 M borate buffer (pH 8.5, 0.1 M boric acid, 25 mM $\text{Na}_2\text{B}_4\text{O}_7$ and 75 mM NaCl) twice for 5 min each, followed by 3 washes in PBS. The cells were then incubated with BrdU Mouse Mab (Bu2a) at a dilution of 1:100 for 1 hr at 37°C with biotinylated secondary antibody (LabVision Cat. TM-060-HL) for 20 min and with streptavidin/peroxidase (LabVision Cat. TA-060-HA) for 30 min at room temperature. Subsequently, sections were subjected to color reaction with 0.002% 3,3'-diaminobenzidine tetrahydrochloride containing 0.005% H_2O_2 in PBS (pH 7.4) and lightly counterstained with hematoxylin.

Cell counting

The number of TUNEL positive stained cells and BrdU labeled cells were quantified by two independent observers in a blind manner. Apoptotic cells were detected by their red colour (Figure 1). Each observer viewed randomly selected 1000 cells in a light microscope at a magnification of 40X. The number of apoptotic cells was determined by apoptotic index (i.e., number of apoptotic cells per 100 cells) and BrdU labeled cells were expressed as the percentage of positive cells (Figure 2). There was no significant difference between the results of two observers ($p=0.23$).

Statistical analysis

Statistical analysis were performed by Kruskal- Wallis nonparametric analysis using SPSS 11.5 (Statistical Programme For Social Sciences, IL, USA). The statistical significance of the difference between the control and hormonotherapy groups was determined by one-way Anova followed by Dunnett T3 test for multiple comparisons. A P value of less than 0.05 was considered significant.

Results

Apoptosis

Figure 3 demonstrates that the TUNEL positive cells count after the 12th hour of the treatment with leuprolide, girelix and their combination with octreotide was higher than the control group. In the grade I EEC cell line the number of apoptotic cell was higher (2.5 ± 0.52) than in the grade II (0.7 ± 0.48) and grade III (0.3 ± 0.48) adenocarcinoma cell line in the drug free group. Apoptotic index in the grade I EEC cell line among girelix-octreotide treated cells (8.5 ± 0.671) and leuprolide-octreotide combination therapy (4.7 ± 0.3) were respectively higher than in the untreated control (2.5 ± 0.167), which was statistically significant ($p < 0.001$, $p = 0.001$). The number of apoptotic cells in the grade II EEC cell line among leuprolide-octreotide (5.5 ± 0.167) and leuprolide (3.1 ± 0.18) were significantly ($p < 0.001$, $p < 0.001$) higher than in the untreated control (0.7 ± 0.153). In the grade III EEC cell line, the number of TUNEL positive cells among leuprolide (4.4 ± 0.819), girelix (4.2 ± 0.389) and girelix-octreotide (3.1 ± 0.277) therapies groups were significantly ($p < 0.001$, $p < 0.001$, $p = 0.002$ respectively) higher than the untreated control (0.3 ± 0.153) (Table 1).

In the presence of 10^{-6} mol/L octreotide, the apoptotic index of the grade I ECC cell line was significantly increased when

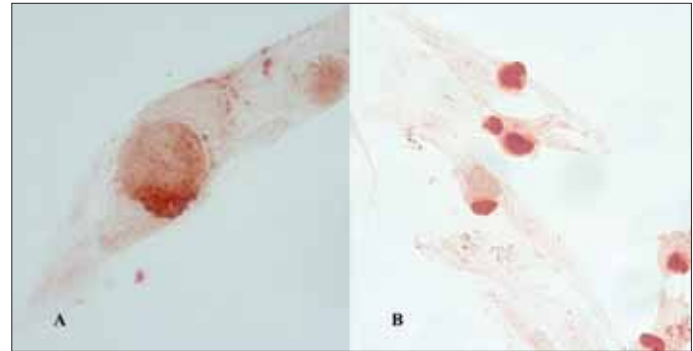


Figure 1 a-b. TUNEL positive cells

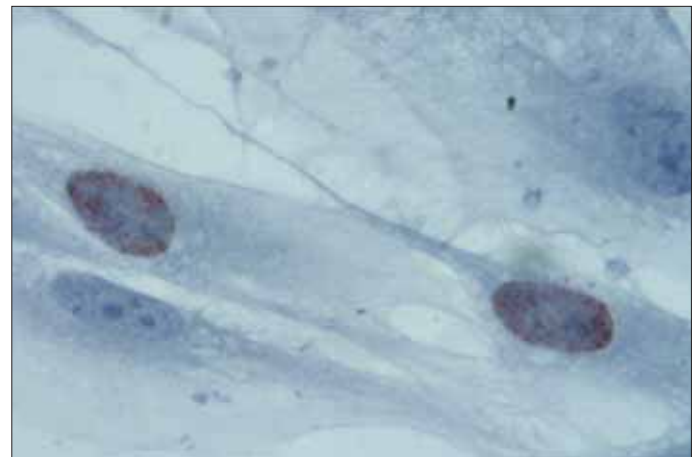


Figure 2. BrdU labeled endometrial cancer cells with light microscopic observation

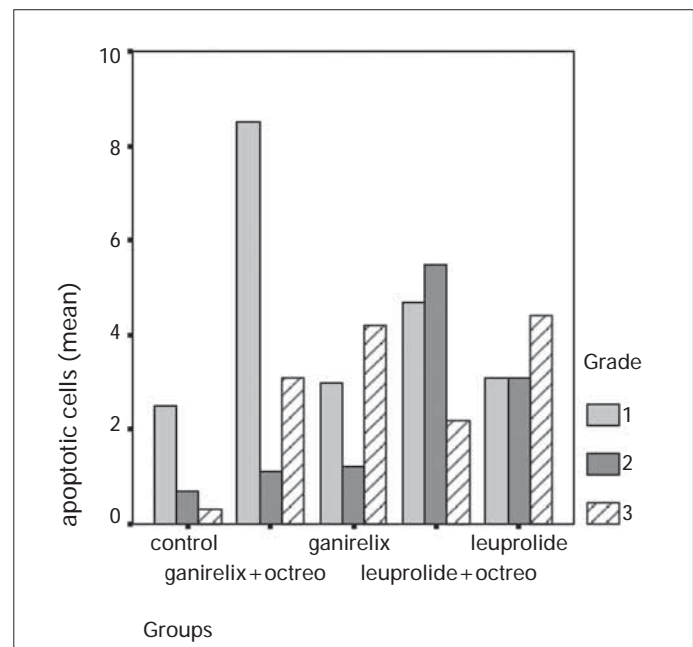


Figure 3. Mean of apoptotic cells in grade I, grade II and grade III endometrial adenocarcinoma, after treatment with GnRH analogues and analogues plus octreotide

compared with the ganirelix and leuprolide added groups ($p<0.001$, $p=0.025$ respectively). However, no additive effect was found with the combination of octreotide on the grade III ECC cell line.

Inhibition of cell proliferation

The inhibitory effect of ganirelix, leuprolide and their combination with octreotide on three different endometrial cancer cells lines (grade I-II-III) was confirmed using BrdU incorporation into untreated and treated EEC cells in vitro at the 24th, 48th and 72th hours. BrdU labeled cells among ganirelix (2.6 ± 0.22), ganirelix and octreotide (3.7 ± 0.26), leuprolide treated cells (3.9 ± 0.1) were respectively lower than the number in untreated controls (7.8 ± 0.36) which was statistically significant ($p<0.001$) at the 24th hour of the study in grade I EEC. The number of BrdU positive cells at the 48th and 72th hours were statistically significant when compared with the controls ($p<0.001$) (Table 2). The antiproliferative response was also seen in the presence of untreated medium in grade II ECC (Table 3). The combination of ganirelix with octreotide did not produce any variation in cell proliferation compared with that obtained with ganirelix alone in grade I and grade II ECC cell lines, while ganirelix and leuprolide alone therapy groups were found more effective than the combination in the grade III EEC cell line (Table 4).

In grade I ECC, when the decreasing percentiles were compared between the groups, the leuprolide plus octreotide treatment group ($p=0.004$) and leuprolide treatment group ($p=0.04$) were found to have significantly higher decrease compared to the control group.

In grade II, no difference was found between the groups. On grade III ECC cell lines, ganirelix plus octreotide ($p<0.001$) and leuprolide plus octreotide ($p<0.001$) had significantly smaller decrease when compared to the control group.

Discussion

GnRH is the primary hypothalamic regulator of reproductive function. Leuprolide acetate is a GnRH agonistic analogue used to treat a wide range of estrogen dependent disorders. It acts on the anterior pituitary, initially inducing a transient rise in gonadotropin release. With continued administration, GnRH causes pituitary desensitization leading to suppressed circulating levels of gonadotropins and estrogens and has been used in the therapy of some sex-hormone-dependent cancers, includ-

ing breast, prostatic, endometrial and ovarian cancer (8-11). GnRH antagonists, unlike the agonistic analogues, do not induce an initial stimulation of gonadotropin release but cause immediate and rapid, reversible and dose dependant suppression of gonadotropin secretion by competitive receptor occupancy of GnRH receptors (12). The antiproliferative effects of GnRH-a seem to be not only through the suppression of gonadal steroids, but also a direct effect on cell growth, and a specific binding site for GnRH has been demonstrated in several tumors responsive to GnRH agonists (5, 13-15). A second type of GnRH receptor has been identified in endometrial and ovarian cancer cells, which transmits a significantly stronger antiproliferative effect than GnRH-I receptor. In mammals, GnRH-II is more widely identified than GnRH-I in periferal tissues, suggesting that GnRH-II may have additional functions (16). In a recent in vitro study, GnRHs treatment was found to cause an increase in integrin $\beta 3$ expression and evoked the activation of focal adhesion kinase (FAK), ERK1/2 and p38 MAPK compared to the control (17). GnRH-II treatment increases the expression of DNA damage-inducible gene 45 (GADD45 α) in endometrial cancer cells (16). GnRH-II induces apoptosis through the binding of GnRH-I receptors, activation of ERK1/2 and p38 MAPK pathways, and induction of GADD45 α signaling. These recent

Table 1. The mean of apoptotic cells in grade I, grade II and grade III endometrial adenocarcinoma, after treatment of GnRH analogues and analogues plus octreotide and their comparisons by groups

	Grade I (mean \pm SE)	Grade II (mean \pm SE)	Grade III (mean \pm SE)
Control	2.5 \pm 0.167	0.7 \pm 0.153	0.3 \pm 0.153
Ganirelix+Octreotide	8.5 \pm 0.671*	1.1 \pm 0.1	3.1 \pm 0.277***
Ganirelix	3 \pm 0.149	1.2 \pm 0.133	4.2 \pm 0.389*
Leuprolide+Octreotide	4.7 \pm 0.3**	5.5 \pm 0.167*	2.2 \pm 0.49
Leuprolide	3.1 \pm 0.18	3.1 \pm 0.18*	4.4 \pm 0.819*
p value	<0.001	<0.001	<0.001
* p<0.001 ** p=0.001 *** p=0.002			

Table 2. Mean of BrdU positive cells in grade I EEC cell line, after treatment with GnRH analogues and analogues plus octreotide, their comparisons by groups and the percentage of decreased proliferative cells

	24 th hour (mean \pm SE)	48 th hour (mean \pm SE)	72 th hour (mean \pm SE)	Decreasing percentage % \pm SE (min-max)
Control	5.6 \pm 0.42	4.8 \pm 0.2	2.7 \pm 0.26	46.3 \pm 10 (-33-71)
Ganirelix+Octreotide	3.7 \pm 0.21	3.2 \pm 0.13	1 \pm 0	71.6 \pm 2.5 (50-75)
Ganirelix	2.6 \pm 0.22	2.4 \pm 0.16	1.1 \pm 0.1	51.6 \pm 8.7 (0-66.6)
Leuprolide+Octreotide	7.6 \pm 0.26	3.4 \pm 0.16	1 \pm 0.14	86.5 \pm 2.1 (71.4-100)
Leuprolide	3.9 \pm 0.1	3.5 \pm 0.16	0.4 \pm 0.16	90 \pm 4.08 (75-100)
p value	<0.001	<0.001	<0.001	<0.001

Table 3. Mean of BrdU positive cells in grade II EEC cell line, after treatment with GnRH analogues and analogues plus octreotide and their comparisons by groups and the percentage of decreased proliferative cells

	24 th hour (mean±SE)	48 th hour (mean±SE)	72 th hour (mean±SE)	Decreasing percentage %±SE
Control	7.2±0.46	1.8±0.35	1.5±0.22	77.8±3.8 (57-90)
Ganirelix+Octreotide	3.7±0.36	0.8±0.13	0.6±0.16	79.1±5.9 (50-100)
Ganirelix	1.9±0.37	0.9±0.1	0.3±0.15	83.3±11.7 (0-100)
Leuprolide+Octreotide	3.1±0.18	1.4±0.22	0.5±0.16	81.6±6.3 (50-100)
Leuprolide	1.5±0.22	3±0	0.2±0.13	85±10.6 (0-100)
p value	<0.001	<0.001	<0.001	0.175

Table 4. Mean of BrdU positive cells in grade III EEC cell line, after treatment with GnRH analogues and analogues plus octreotide and their comparisons by groups and the percentage of decreased proliferative cells

	24 th hour (mean±SE)	48 th hour (mean±SE)	72 th hour (mean±SE)	Decreasing percentage %±SE (min-max)
Control	5.5±0.16	5.5±0.56	2.2±0.29	59.6±5.84 (20-80)
Ganirelix+Octreotide	3.8±0.24	3.3±0.21	3±0.29	16±11 (-50-75)
Ganirelix	5.2±0.41	2.4±0.16	2.1±0.1	56.4±5 (25-71.4)
Leuprolide+Octreotide	4.5±0.26	2.3±0.3	2.4±0.22	44.1±6.8 (0-80)
Leuprolide	7.5±0.26	7.2±0.24	1.4±0.22	81.4±2.5 (66.6-88.8)
p value	<0.001	<0.001	<0.001	<0.001

studies demonstrated that GnRH related inhibition of endometrial cancer cell proliferation is mediated by GnRH receptors and also related with mitogenic signal transduction molecules. Ganirelix is a synthetic third generation gonadotropin-releasing hormone antagonist that blocks GnRH receptors in the anterior pituitary gland, preventing endogenous GnRH from inducing LH and FSH release. GnRH antagonists have agonistic effects on this type II receptor (18). GnRH II has a strong antiproliferative effect without involving induction of apoptosis. It could be speculated that the additional receptors interact with pathways regulating the cell cycle (19).

Previous in vitro studies have shown that the number of Ishikawa endometrial cancer cells was reduced by the GnRH-I antagonist cetrorelix (SB-75) in a dose-dependent manner (13). This growth inhibitory effect of SB-75 was not found to be associated with a decrease in the number of cells in the S phase but was associated with an induction of apoptosis (13). The decrease in the rate of apoptosis in grade 3 adenocarcinoma in the drug free group may reflect loss of cell homeostasis control and decreased differentiation. It may stated that there are tissue-specific differences controlling the progression of cancer cells.

Heterogeneity of receptor density appears to be common in endometrial adenocarcinomas as the vast majority of tumors showed substantial receptor heterogeneity of both ER and PR within the tumors. Hormone receptor heterogeneity of endometrial carcinoma has been discussed also in the context of the primary tumors and the metastases having different hormone receptors status (20). In a recent study, Jeon et al demonstrated that GnRH receptor expression was not related to the histotype

of endometrial cancer, disease stage, tumor differentiation, lymph node metastasis and myometrial invasion (7). In invitro conditions, GnRH analogues will show their effect via GnRH receptors. As previously described, GnRH agonists can induce Fas Ligand production in GnRH receptor bearing endometrial carcinoma. The Fas Ligand expression linked to the GnRH receptor activation may mediate the antiproliferative action of GnRH agonist by increasing apoptosis within the cancer cells, but the GnRH effect was abolished by the addition of the antagonist antide (21). In this presented study, apoptosis was found to be induced in both agonist and antagonist application. The cell culture studies demonstrated an increase in the programmed cell death in the grade I, II and III endometrial adenocarcinoma cell lines at the 12th hour of the treatment groups. However, the proliferation index varied depending on the histological grade, which may contribute to the difference in tumor behavior. These apoptotic and antiproliferative effects occur at micromolar concentrations. In fact, when administered subcutaneously, plasma concentrations of leuprolide at therapeutic doses are in the nanomolar range (22). Also, blood concentrations of antagonist analogues ranged between 30-60 ng/ml with an injected dose of 10 mg/day (18). Previous dose response experiments showed the antiproliferative effect of the lower concentration of the GnRH analogues in the HEC-1a lines and Ishikawa cell lines (13, 23). Moreover, primary in vitro cultures are closer to in vivo biology when compared to cancer cell lines. Our results suggest that in endometrial cancer, GnRH is part of a negative autocrine system. This knowledge could help clinicians decide whether to use GnRH agonistic or antagonistic

analogue and a combination with octreotide to inhibit cell proliferation, in patients with recurrent and/or advanced disease. However, further studies are needed to evaluate the possible additive roles of somatostatin analogues in endometrial cancer. The insulin-like growth factor-1 (IGF-1) signalling pathway has important roles in regulating cellular proliferation and apoptosis. In vivo carcinogenesis models indicate that high levels of plasma IGF-1 are associated with increased risk of cancer (24). Therapeutic strategies that target the IGF-1 receptors or reduce serum levels of growth factor and IGF-1 may be important for the antineoplastic activity of cancer dependent IGF pathway (25). Many human tumors can express somatostatin receptors. In a series of 28 randomly selected endometrial carcinomas, sst₁ was present in 32%, sst_{2A} in 39%, sst₃ in 43%, sst₄ in 4% and sst₅ in 4% of cases. 36% expressed more than one sst receptor (26). Somatostatin analogue octreotide (SMS 201-995) bind with a high affinity for somatostatin receptors 2 and 5 but show a relatively low affinity for sst₃ (27). Receptor positive endometrial carcinomas may be a potential target for somatostatin analogues. There are few reports on the use of GnRH-a and somatostatin analogues in Gynecologic Oncology. The use of octreotide in gynecologic tumors is reported as case reports. Preclinical studies on tumor biology on somatostatin and its receptors are still under research. As in vitro studies do not parallel the in vivo milieu, these studies are not yet conclusive. Our study reports the effects of GnRH agonist, antagonist and octreotide in three different grade endometrium cancer cell lines. They were compared in terms of their effects on apoptosis and proliferation. In summary, the current study suggests that GnRH agonistic and antagonistic analogues and their combination with octreotide induce apoptosis. This may be a part of a therapeutic mechanism. Since we did not evaluate the GnRH receptor expression and the molecular pathway of apoptosis in the presented study, the extent of growth inhibition may be affected by more precisely targeted application of GnRH analogues on appropriate GnRH receptor positive cells. Further studies would help provide a better understanding of the molecular mechanisms of apoptosis and cell cycle dynamics.

Conflict of interest

No conflict of interest is declared by authors.

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