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Comparison of the effects of cetrorelix, a GnRH antagonist, and leuprolide, a GnRH agonist, on experimental endometriosis

Devrim Altintas[1], Arif Kokcu[1], Migraci Tosun[1], Mehmet B. Cetinkaya[1] and Bedri Kandemir[2]

Departments of [1]Obstetrics and Gynecology and [2]Pathology, School of Medicine, University of Ondokuz Mayis, Samsun, Turkey

Abstract

Aim: In the present study, we aimed to compare the effects of cetrorelix and leuprolide on endometriosis.

Methods: This randomized, placebo-controlled, single-blind, experimental study was performed on 45 Wistar adult female rats in the Experimental Surgery Laboratory at Ondokuz Mayis University. After the peritoneal implantation of endometrial tissue, rats were randomized to three equal intervention groups: (i) control group, (ii) leuprolide group, and (iii) cetrorelix group. Six weeks later, following implant volume measurements (volume-1) by performing a second laparotomy, saline (0.1 cc/rat) was administered subcutaneously to the control group once a week, leuprolide (0.075 mg/kg) subcutaneously to the leuprolide group twice at 4-week intervals and cetrorelix (0.001 mg/rat/day) subcutaneously to the cetrorelix group for 8 weeks. At the end of the treatment, by performing a third laparotomy, implant volumes were remeasured (volume-2) and implants were totally excised for histopathological examination. The volume-1 and volume-2 values within the groups, and stromal and glandular tissue scores between the groups were compared.

Results: In both the leuprolide group and the cetrorelix group, volume-2 as compared to volume-1 had significantly reduced ($P < 0.01$, $P < 0.01$ respectively), while there was no significant volume change in the control group ($P > 0.05$). In this group, when compared with the control group, glandular and stromal tissues had significantly lessened ($P < 0.01$, $P < 0.01$ respectively).

Conclusion: Leuprolide and cetrorelix were found to have similar efficacy in the regression of both the size and the histological structure of experimental endometriotic implants.

Key words: cetrorelix, experimental endometriosis, leuprolide, rat.

Introduction

Endometriosis is an enigmatic disease found in as many as 30% of reproductive-aged women. It is seen in 10% of hysterectomy surgeries, in 16–31% of laparoscopies, and in 53% of adolescents with pelvic pain severe enough to warrant surgical evaluation. The symptoms for women who suffer from this malady vary but may include subfertility or chronic pelvic pain. Because endometriosis lesions rely on estradiol for growth, most of the existing drug regimens work by creating hypoestrogenism. Current medical therapies rely on the interruption of normal cyclic, ovarian hormone production resulting in an environment not

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Reprint request to: Professor Arif Kokcu, Bahcelievler mah, Abdülhakhamid Cad, Onursal Ap, No: 19/4, 55070 Samsun, Turkey.
Email: arifkokcu@yahoo.com

conducive to the growth of endometriosis. The current accepted medical therapies for endometriosis include danazol, progestational agents, oral contraceptive agents, and gonadotropin-releasing hormone analogues which all function similarly in relieving pain.[1–3]

Clinical applications of gonadotropin-releasing hormone (GnRH) agonists are based on gradual down-regulation of pituitary receptors for GnRH, which leads to the inhibition of the secretion of gonadotropins and sex steroids. Moreover, GnRH agonists are assumed to act on immune cells, inducing a suppression of cytokine levels. Cytokines and growth factors derived from peritoneal immune cells regulate the growth of endometriosis implants.[4–6] It has been shown that GnRH analogues enhanced apoptosis in the endometrial epithelial cell, and this was accompanied by an increase in expression of the pro-apoptotic proteins Bax and Fas-Ligand (FasL) and a decrease in expression of the anti-apoptotic protein Bcl-2. GnRH analogues are indicated for clinical situations in which the suppression of endogenous gonadotropins (precocious puberty, contraception and controlled ovarian hyperstimulation) or sexual steroids (endometriosis, prostate hyperplasia, cancer and uterine fibroids) is desired. GnRH antagonists immediately block pituitary GnRH receptors and, therefore, achieve rapid therapeutic effects. It has been reported that all of the current indications for GnRH agonist desensitization may prove to be indications for a GnRH antagonist, including endometriosis, leiomyoma and breast cancer in women, benign prostatic hypertrophy and prostatic carcinoma in men, and central precocious puberty in children. However, the best clinical evidence has been in assisted reproduction and prostate cancer.[4,7–9] To date two peptidic GnRH antagonists have been launched for use in female infertility promoting regimens. Cetrorelix has been developed by ASTA Medica and Zentaris for the treatment of infertility regimens in *in vitro* fertilization and assisted reproductive technology and is currently marketed by Serono for these indications. Zentaris has also evaluated Cetrorelix in a range of other disease areas such as uterine fibroids, endometriosis, benign prostatic hyperplasia (BPH) and breast, ovarian and prostate cancers in various long acting formulations.[8,9]

The MEDLINE database was reviewed for English-language articles comparing the effects of GnRH agonists and antagonists on the size and histological structure of endometriosis and any experimental or clinical study comparing the effects of GnRH agonists and antagonists couldn't be found. So, this randomized, placebo-controlled, single-blind, experimental study was planned to compare the effects of cetrorelix, a GnRH antagonist, and leuprolide, a GnRH agonist, on the size and histological components of experimental endometriosis in rats.

Materials and Methods

The present study was performed in the Surgical Research Center of the University of Ondokuz Mayis with the approval of the University of Ondokuz Mayis Animal Ethics Committee. In the study, 45 adult female rats of Wistar-Albino race were used. Laparotomy was carried out on the rats through a 3 cm abdominal vertical incision. The $0.5 \times 0.5 \times 0.1$ cm piece taken through microscissors from the uterine horn was implanted with a single suture using 6/0 vicryl onto abdominal peritonea. The rats were randomized to three equal intervention groups with 15 rats in each group: (i) control group, (ii) leuprolide group, and (iii) cetrorelix group.

Six weeks after the first laparotomy, a second one was performed to evaluate whether the liveliness of the implant continued and then the implant was transformed into a cystic structure (Fig. 1). The implant

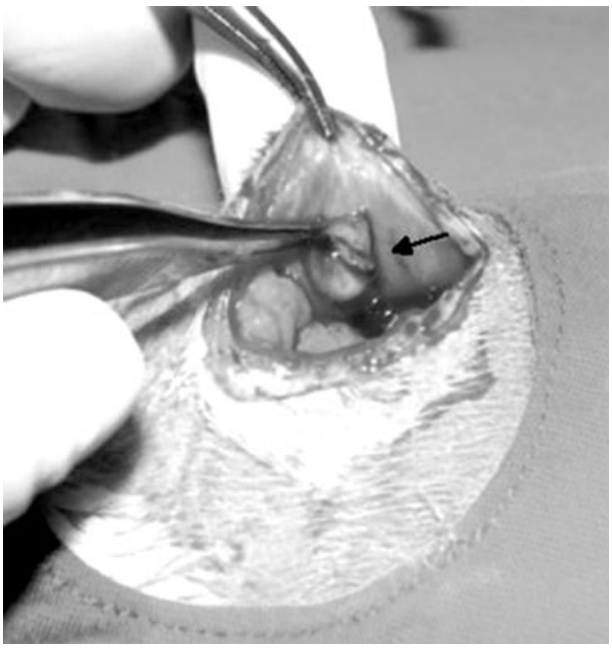

Figure 1 Cystic formation (Volume-1) on the abdominal parietal peritoneum of a rat, 6 weeks after the implantation of endometrial tissue. Arrow shows a vascularized cystic formation.

Figure 2 An atrophic endometriotic implant (Volume-2) at the end of treatment in the cetrorelix group.

volumes were calculated by measuring their dimensions (length, width, height) by micrometer (Volume-1). For volume calculation, an ellipsoid volume formula ($\pi/6 \times$ length $\times$ width $\times$ height) was used. Following the volume calculation, saline (0.1 cc/rat) was administered subcutaneously to the control group once a week, leuprolide (0.075 mg/kg) subcutaneously to the leuprolide group twice at 4-week intervals and cetrorelix (0.001 mg/rat/day) subcutaneously to the cetrorelix group for 8 weeks. At the end of 8-week treatment, by performing a third laparotomy, the implant volumes were remeasured (Volume-2) (Fig. 2). In order to examine, histopathologically, the amount of stromal tissue (ST) and glandular tissue (GT) in the implants, they were totally excised. Those performing the measurements of volume-1 and volume-2 were not aware of the treatment arms.

After measuring the volumes of the endometrial implants on the third laparotomy, these implants were totally removed and stabilized in 1% formaldehyde solution for histopathological examination. The median values of volumes 1 and 2 were compared in the same group. Histopathologically, the amounts of ST and GT in the implants were examined and scored. The histopathological examination and scoring were performed by a pathologist who was not aware of the treatment arms.

Biopsy samples were all fixed in 10% buffered neutral formaline for a minimum of 12 h. After routine procedures, specimens were embedded in paraffin and cut into 6 micron meter sections. The sections stained with hematoxylin and eosin and Gomori's one-step trichrome stain were used to evaluate the GT and the ST. ST scoring was performed according to the percent of 10 high-power field containing ST on microscopic examination, and GT scoring according to the number of secretory glands (Table 1).

Table 1 The scoring system used in the evaluation of glandular activity and stromal tissue in the endometriotic implants on microscopic examination

Score	GT (gland number per 10 hpf)	ST (percent of 10 hpf containing ST)
0	Absent	Absent
1	1	<25
2	2–3	25–50
3	≥4	>50

GT, glandular tissue; ST, stromal tissue.

The data distribution was confirmed if it was normally distributed by using the Shapiro Wilks Normality test. As the distribution of both the volume values and the scores of histological parameters was found not to be normal, the nonparametric Mann-Whitney U test was used to compare the group differences of GT and ST scores; also, within each group the values of the volumes 1 and 2 were compared using the Wilcoxon Signed Ranks Test. The data were presented as the median, maximum and minimum and $P < 0.05$ value was accepted as statistically significant.

Findings

Pre-treatment and post-treatment body weights of the rats ranged between 210 and 230 g. In the second laparotomy, it was seen that the endometriotic implants in all the rats had changed into vascularized, cystic structures (Fig. 1). The median values (median [minimum, maximum]) of volume-1 in the control, leuprolide and cetrorelix groups were 80.0 (48.0–100.0), 80.0 (64.0–120.0) and 80.0 (64.0–100.0) respectively (The mean ± standard deviation (SD): 80.26 ± 18.04, 80.26 ± 19.15, and 80.8 ± 11.82 mm^3 respectively). The median values of volume-1 didn't show a statistically significant difference between the groups ($P > 0.05$). The median values of volume-2 in the control, leuprolide and cetrorelix groups were 120.0 (48.0–168.0), 8.0 (2.0–64.0), and 4.0 (1.0–36.0) respectively (The mean ± SD: 103.06 ± 45.95, 13.60 ± 19.93, and 7.80 ± 8.75 mm^3 respectively). When the volume-1 and volume-2 were compared in the same group, the

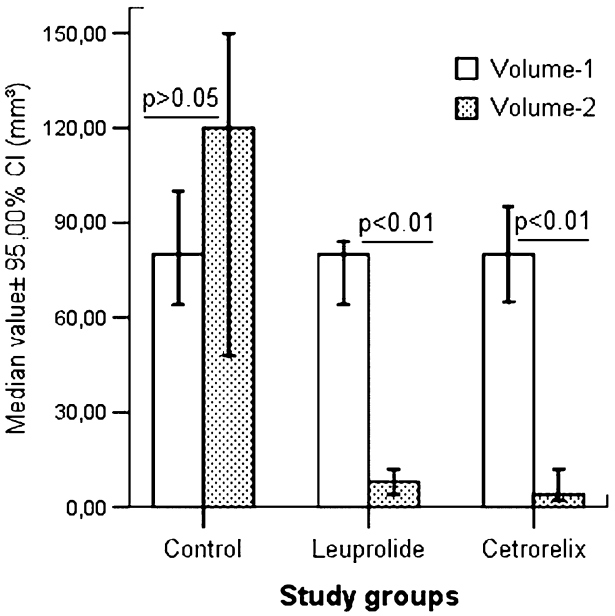

Figure 3 Comparison of the pre-treatment (Volume-1) and the post-treatment (Volume-2) endometriotic implant volumes in the study groups.

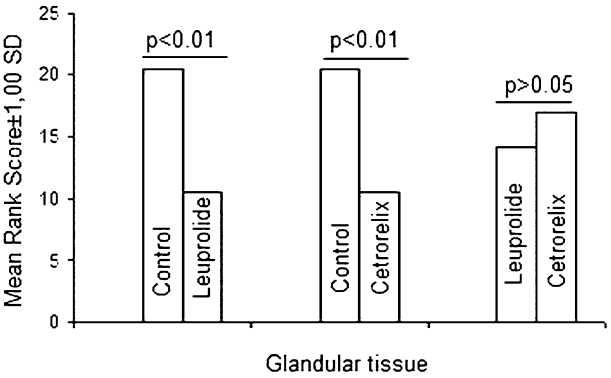

Figure 4 Comparison of the mean rank scores of the post-treatment glandular tissue (GT) in the endometriotic implants between the study groups.

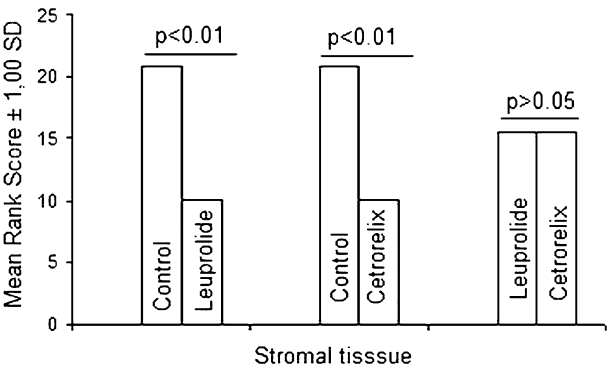

Figure 5 Comparison of the mean rank scores of the post-treatment stromal tissue (ST) in the endometriotic implants between the study groups.

volume decreases in the leuprolide and cetrorelix groups were found to be statistically significant ($P < 0.01$, $P < 0.01$ respectively) (Fig. 2), while there is no statistically significant difference between volume-1 and volume-2 values in the control group ($P > 0.05$) (Fig. 3).

In the histopathological evaluation of the endometriotic implants, the median values of GT scores in the control, leuprolide and cetrorelix groups were 2.0 (1.0–3.0), 0.0 (0.0–3.0), and 1.0 (0.0–3.0), respectively (Mean ± SD: 1.86 ± 0.74, 0.60 ± 1.12 and 0.73 ± 0.88, respectively). The median values of ST scores in the same groups were 1.0 (1.0–3.0), 1.0 (0.0–1.0), and 1.0 (0.0–1.0) respectively (Mean ± SD: 1.60 ± 0.73, 0.53 ± 0.51, and 0.53 ± 0.51 respectively). When compared according to Mean Rank Scores, both the ST and GT scores in the leuprolide and cetrorelix groups were found to have decreased as compared to the control group ($P < 0.01$ and $P < 0.01$, respectively) while there is no statistically significant difference between the GT and ST scores of the leuprolide and cetrorelix groups (Figs 4,5).

Discussion

Gonadotropin-releasing hormone is a decapeptide hypothalamic hormone that acts upon 7-trans membrane spanning GnRH receptors in the pituitary. This action leads to the secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) that in turn act on the reproductive organs, regulating gonadal steroid production, spermatogenesis and follicular development. Peptidic agonists of the GnRH receptor have been known for many years and are currently employed therapeutically in the treatment of prostate and breast tumors, uterine fibroids, precocious puberty, endometriosis, premenstrual syndrome, contraception and infertility.[4,8,9] Concomitantly to the development of GnRH-agonists, GnRH derivatives with high binding affinity to the pituitary receptor were identified, which did not cause but inhibited release of gonadotropins immediately upon receptor binding. GnRH-antagonists bind to the pituitary GnRH receptor, but are not functional in inducing GnRH receptor cross-linking, a process that

appears to be necessary to affect gonadotropin release. These analogues exert an antagonistic effect by competing with endogenous GnRH for pituitary binding sites. Because of the lack of any intrinsic activity of these compounds, the characteristic initial 'flare-up' effect of GnRH-agonist administration is absent. Currently, GnRH-antagonists are used clinically mostly for the indication of premature LH surge prevention in the context of controlled ovarian hyperstimulation for assisted reproduction. By employing GnRH-antagonists for controlled ovarian hyperstimulation (COH), a more rapid suppression of gonadotropin release can be achieved, enabling shorter treatment regimes for ovarian stimulation as compared to the gold standard in ovarian hyperstimulation, the so-called long protocol utilizing agonistic GnRH-analogues.[7–11]

At this time, only three molecules are available. Two GnRH-antagonists are commercially available for use in COH. Another third-generation GnRH-antagonist, abarelix, has recently been approved by the FDA for the palliative treatment of advanced prostatic cancer. A number of further GnRH-antagonists, both peptide and non-peptide, has been identified, but has as yet not attained marked approval or entered the stage of clinical testing. Cetrorelix is mainly used for in vitro fertilization protocols, but research is currently being carried out in benign prostatic hypertrophy. Cetrorelix also seems to be useful in the treatment of endometriosis which, in most cases, is an estrogen-dependent disease. Furthermore, fewer side effects occur with this agent (e.g. postmenopausal symptoms) and no estradiol add-back is needed. Cetrorelix is commercially available as a 0.25-mg preparation for daily subcutaneous (s.c.) injections and as a 3 mg s.c. intermediate depot preparation. Mean terminal half-life of a single s.c. dose of 0.25 mg is 20 h for cetrorelix. Subcutaneously injected cetrorelix of 3 mg has a mean terminal half-life of 63 h.[9–12]

The objective of long-term treatment plus hormone add-back is to minimize bone loss without compromising the efficacy of relief of pain. The so-called estradiol threshold theory provides the rationale for this approach. It has been proposed that estrogen within a certain concentration range may prevent bone loss while hindering the growth of ectopic endometrial lesions. The estrogen threshold that is associated with clinical improvement and minimal side effects due to hypoestrogenism may vary between estrogen-dependent diseases. For endometriosis, the efficacy of GnRH-agonist induced hypogonadism plus steroid add-back has been confirmed by a number of randomized controlled clinical trials. The efficacy of GnRH-agonist treatment has not been diminished by the addition of exogenous steroids and it has been suggested that estradiol levels <40 pg/mL over a longer time period are required for hindering the proliferation of ectopic endometrium, while 20–40 pg/mL estradiol serum level might be sufficient to reduce the risk of bone loss and to avoid postmenopausal symptoms. Because of the dose-dependent effect of GnRH antagonists on endogenous LH/FSH secretion, the estradiol level can possibly be "titrated" into the desired range, which is comparatively difficult with GnRH agonists that usually induce a completely downregulated status after the initial flare-up phenomenon. However, also for GnRH-agonists, decreasing the dosage administered after an initial period of pituitary downregulation has been suggested as effective while reducing the side effects of downregulation. With antagonists, immediate decrease in estrogen levels can be expected after administration, and therefore an earlier onset of the therapeutic effect (pain relief) can be hypothesized. Both effects might prove useful with respect to endometriosis treatment strategies: intermittent reinduction therapy might be more efficient in a shorter time course and long-term therapy might be possible without the cost and hassle of exogenous steroid supplementation.[7–9] Peptidic GnRH antagonists have found limited clinical use to date and are currently only indicated for use in the treatment of infertility although they are also in advanced trials for prostate cancer. The disadvantage of the initial stimulation by GnRH agonists is overcome by utilizing GnRH antagonists as they act directly to inhibit gonadotrope function. In addition dose titration of peptide antagonists may be explored more readily to allow partial suppression of gonadal steroids to levels sufficient to ameliorate disease (such as in endometriosis and uterine fibroids) without any hypo-estrogenic side-effects.[9–13]

In the present study, post-treatment endometriotic implant volume in the leuprolide group and cetrorelix group, compared to pre-treatment volume, had reduced statistically significantly ($P < 0.01$, and $P < 0.01$ respectively), which shows clearly that there is a similar regressing effect of both drugs on experimental endometriotic implants (Fig. 3). GT and ST that develop by the influences of estrogen and progesterone hormones are the most important histological criteria of the endometriosis formation. The decrease in these parameters is a histological indicator of the regression in the endometrial tissue.[14,15] In the current study, the

fact that GT and ST scores in both the leuprolide group and cetrorelix group were found to be significantly lower than that of the control group ($P < 0.01$ and $P < 0.01$ respectively) shows a significant regression in the endometriotic implants histologically (Figs 4,5). Being statistically similar of both the volume regression and histological regression of the endometrial implants in the leuprolide and cetrorelix groups is an important result. To the best of our knowledge, this is the first study comparing the effect of leuprolide and cetrorelix on the size and the histological parameters of the experimental endometriosis.

In conclusion, the findings of the present study show that leuprolide, a GnRH agonist, and cetrorelix, a GnRH antagonist, are equally effective at regressing both the size and the histological components of experimental endometriosis. Besides this, the study has experimentally proved that cetrorelix could be considered as an alternative choice instead of GnRH agonists for the treatment of endometriosis. The most important advantages of cetrorelix are that it has fewer side effects (e.g. postmenopausal symptoms) and no estradiol add-back is needed.

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