Therapeutic Efficiency of Tibolone in a Rat Endometriosis Model

Bir Sıçan Endometriozis Modelinde Tibolonun Terapötik Etkinliği

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ABSTRACT Objective: This study aimed to evaluate the therapeutic efficiency of tibolone in an experimental rat model of intra-abdominal endometriosis. **Material and Methods:** In this experimental study, intra-abdominal endometriosis was induced in 30 female rats surgically. Four weeks after this procedure, laparotomy was performed again. The dimensions of the endometriosis foci were recorded and right after, the operation was completed. Rats were randomly divided into three groups and subsequently, the treatment was started. In the first group (n= 8), a single dose of 1 cc 0.9% NaCl was injected subcutaneously. In the second group (n= 8), intramuscular injection of 1 mg leuprolid acetate was administered. In the third group (n= 8), 1 mg/kg/day of tibolone was given by gavage. At the end of 4- weeks of drug administration, laparotomy was performed to all rats. The dimensions of the endometriosis foci were recorded. Afterwards, all the rats were sacrificed. The differences in the areas of endometriotic implants and adhesion scores were compared between the groups. **Results:** The dimensions of the endometriosis foci in the groups treated with leuprolid ($p< 0.05$) and tibolone ($p< 0.05$) were significantly diminished compared to that of the control group. No statistically significant differences were found between the treatment groups. **Conclusion:** In a rat endometriosis model, tibolone has a similar efficiency as that of leuprolid acetate, an agent used for conventional medical treatment of endometriosis. With its androgenic and progestagenic characteristics, tibolone deserves attention as an alternative agent in the medical treatment of endometriosis in human.

Key Words: Rats; endometriosis; gonadotropin-releasing hormone; tibolone

ÖZET Amaç: Bu çalışmada karın içi endometriozisli deneysel sıçan modelinde tibolonun terapötik etkinliğinin değerlendirilmesi amaçlandı. **Gereç ve Yöntemler:** Bu deneysel çalışmada, 30 dişi sıçanda karın içi cerrahi endometriozis meydana getirildi. Bu işlemden dört hafta sonra tekrar laparotomi yapıldı. Endometriozis odaklarının boyutları kaydedildi hemen ardından operasyon tamamlandı. Sıçanlar rastgele üç gruba ayrıldı ve daha sonra tedavi başlandı. Birinci gruba (n= 8), NaCl 1 cc %0.9 tek doz olarak cilt altına enjekte edildi. İkinci gruba (n= 8), leuprolid asetat 1 mg intramusküler olarak uygulandı. Üçüncü gruba (n= 8), tibolon 1mg/kg/gün olarak gavaj ile verildi. Dört hafta ilaç alımının sonrasında tüm sıçanlara laparotomi yapıldı. Endometriozis odaklarının boyutları kaydedildi. Daha sonra tüm sıçanlar öldürüldü. Gruplar arasında endometriotik implantlar ve adezyon skoru alanlarındaki farklılıklar karşılaştırıldı. **Bulgular:** Leuprolid ($p< 0.05$) ve tibolon ($p< 0.05$) ile tedavi gruplarında endometriozis odaklarının boyutları kontrol grubuna göre anlamlı olarak azalmıştı. Tedavi grupları arasında istatistiksel olarak anlamlı fark yoktu. **Sonuç:** Sıçan endometriozis modelinde, tibolonun endometriozis tedavisinde konvansiyonel tıbbi tedavinin bir ajanı olan leuprolid asetata benzer bir etkinliği vardır. Androjenik ve progestajenik özellikleri ile tibolon insan endometriozis tedavisinde alternatif bir ajan olarak dikkat çekmektedir.

Anahtar Kelimeler: Sıçanlar; endometriyozis; gonadotropin salgılatıcı hormon; tibolon

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Endometriosis is a chronic disease characterized by the presence of the endometrial gland and stroma out of the endometrial cavity. It is mainly a disease of the women of reproductive age and its estimated incidence is 10%.[1,2] Various theories on its etiology such as retrograde menstruation and direct implantation, vascular invasion, lack of immune response, and genetic factors have been proposed. However, the exact etiology has not been defined yet.[1-4]

Endometriosis is an estrogen dependent disease. These foci involve estrogen receptors and estrogenic stimulation increases the development of endometriosis foci.[1,2]

Various agents [progestins, gonadotropin releasing hormone analogs (GnRHa), combined oral contraceptives, danazol] used in the medical treatment of endometriosis show their effects by anti-estrogenic activity.[5] The treatment of endometriosis was investigated in earlier studies with a rat model using GnRHa and gonadotropin releasing hormone antagonists and various agents such as pentoxiphylline, doxycycline, and levamizol.[5-9] GnRH analogues are being used effectively in the treatment of endometriosis and there is a consensus in the current literature that these agents are effective.[5,9] The use of GnRHa with monthly injections or injections with 3-month intervals inhibits ovarian estrogen synthesis and forms a hypoestrogenic environment, thus diminishing the endometriosis foci.[5,10]

Tibolone is a molecule that has a selective regulatory effect on the estrogen receptors.[11] It is rapidly metabolized and converted into three active metabolites. Three alpha hydroxyl (OH) and 3-beta-OH metabolites show an estrogenic activity on the bones, vagina, and central nervous system.[11,12] Delta-4 metabolite, however, has progestagenic and androgenic characteristics and inhibits the proliferation of endometrial cells.[11,12] It is mainly used as an alternative to the postmenopausal hormone therapy. This study aimed to evaluate and compare the effects of tibolone and GnRHa on an experimental endometriosis model in the rats.

MATERIAL AND METHODS

The present study was conducted at the Experimental Animals Laboratory of the surgery department of Gulhane Military Medical Academy, between the July 04, 2009 and September 18, 2009. The approval of the institutional ethics committee was obtained before the study and all the procedures on the animals were conducted in accordance with the directions of '*Guide for the Care and Use of Laboratory Animals*'. The study was performed on 30 female Swiss-Albino rats that weighed 200-250 grams, were in the proestrus phase and non-pregnant. The rats were kept in steel cages in a climate-controlled environment under ad libitum feeding conditions and provided with access to daylight between 06:00 am and 6:00 pm. The study had three phases, the details of which are presented below:

PHASE I

The endometriosis model in the rats was formed by the method previously described by Vernon and Wilson:[13]

All the rats were administered 100 mg/kg of ketamin hydrochloride (Ketalar® flakon) for anesthesia. The trichotomy of the abdominal region was made by electric razor. A midline incision of 3 cm was made after disinfection of the abdomen with iodine while the rats were in supine position. The cutaneous, subcutaneous and muscle tissues were separated and thus, the peritoneal cavity was accessed. A 15 mm segment of the right uterine corn was removed. This cylindrical segment was opened with longitudinal incision and the endometrial layer was exposed. The endometrial face of the removed segment was sutured with a single suture to mesentery using non-absorbable suture material (No:4/0 silk suture). The layers of the abdomen were consecutively closed, and the procedure was ended.

PHASE II

Twenty-eight days after the first operation, the rats underwent a second laparotomy under sterile conditions as described above. The intra-abdominal endometriotic foci and adhesions were observed. The length and width of the implants were measured and the surface area of the implants was calculated.

The adhesions were classified according to the scoring system of Blauer, which was used previously in rat endometriosis models[14,15] as follows:

ADHESION SCORE

0: No adhesion

1: Thin adhesions

2: Thick adhesion in one area

3: Widespread thick adhesions

4: Adhesions of the internal organs to the abdominal wall

The abdomen was closed and the procedure was ended. Three rats with no suspected endometriosis foci macroscopically and three rats that died in the first phase were excluded from the study. The remaining 24 rats were divided into three groups.

Three days after the second phase, the rats in the first group (the control group, n= 8) were injected with 1 cc of 0.9% NaCl.

The rats in the second group (n= 8) were administered 1 mg intramuscular leuprolid acetate (Lucrin Depot- 3M®, Abbott), as was used in previous studies as the treatment dose.[5,9]

The rats in the third group (n= 8) were given 1 mg/kg/day of tibolone (Livial® tablet, Organon) for four weeks with gavage. The dose of tibolone was calculated based on the recommendations by Genazzani et al. with the therapeutic dose range (0.2-2 mg/g/day) arranged for female rats.[16]

PHASE III

All rats were sacrificed by cervical dislocation and were subjected to laparotomy procedure as defined earlier. The area of the endometriotic foci and intra-abdominal adhesions were recorded.

The changes in the area of endometriosis foci of all groups were measured and compared. The intra-abdominal adhesion scores were also compared.

STATISTICAL ANALYSIS

The data were evaluated using SPSS 15.0 program. All the data were expressed as mean values. The intergroup comparisons were made wit paired sample t test. A p value <0.05 was considered as statistically significant.

RESULTS

During the study three rats died and another three rats with no suspected endometriosis foci macroscopically were excluded from the study. The study was completed with 24 rats.

In the second phase, the mean area of endometriotic foci and intra-abdominal adhesion scores among the groups were similar (Table 1). Endometriotic foci were macroscopically observed as cystic or vascular lesions. At the end of the treatment, the area of the endometriotic foci and adhesion scores were found to decrease in all three groups (Figure 1). In Groups 2 and 3, the reduction was more marked compared to that in the control group and the difference was statistically significant (p< 0.05) (Table 2).

DISCUSSION

Novel treatment methods of endometriosis, a chronic disease with unclear etiology, are frequently used in experimental rat, mice, monkey, and rabbit models. One of these methods was used by Vernon and Wilson for the first time in a rat endometriosis model because rats are inexpensive and have a short 4-day menstrual cycle, short luteal phase of the cycle, and predominant estrus phase.[13,17]

GnRHa is widely used in the medical treatment of endometriosis with a proven efficiency.[18-

TABLE 1: Pre- and posttreatment area of endometriotic foci and adhesion scores in the three groups.

	Area of endometriotic foci (mm^2)			Adhesion score		
	Pre	Post	P value	Pre	Post	P value
Control (n= 8)	36.6 ± 11.5	28.8 ± 7.8	0.070	3.1 ± 0.8	1.6 ± 0.5	0.005
Leuprolid (n= 8)	35.2 ± 9.9	9.3 ± 8.9	0.003	2.8 ± 0.8	1 ± 1.06	0.004
Tibolone (n= 8)	33.1 ± 12.1	15.3 ± 8.2	0.000	2.5 ± 0.9	1.1 ± 1.1	0.001

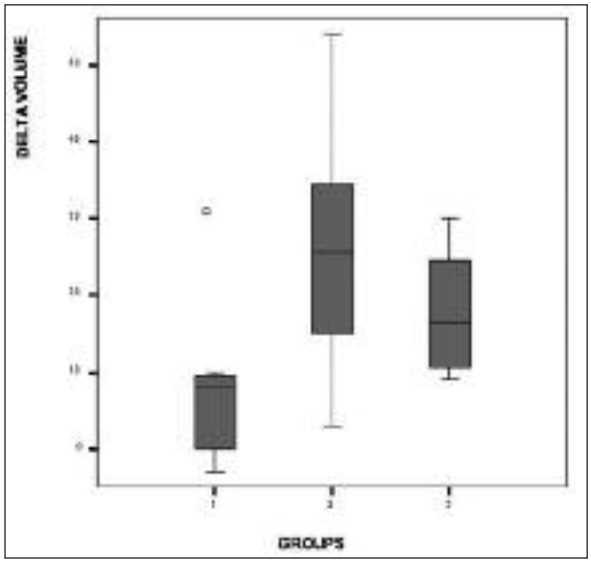

FIGURE 1: Comparison of delta values (postreatment-pretreatment) of areas of endometriotic foci among the three groups.

[20] In the use of GnRHa, FSH and LH secretion from the hypophysis are temporarily increased. However, with its supraphysiological dose, agonistic activity is sustained, receptors are down-regulated, and hypophysis-gonad axis is inhibited. In consequence of inactivation of the oral form, it is used in the form of subcutaneous or intramuscular injections. GnRHa has been shown to induce atrophy in the endometriotic foci, increase the activity of peritoneal natural killer cells, and reduce the levels of peritoneal IL-6, IL-1β, TNF α, and Ca-125 in rat endometriosis models.[21,22] In our study, posttreatment macroscopic changes and adhesion scores were evaluated in a rat endometriosis model. Parallel to the findings in the literature, the group that received GnRHa treatment had significant reductions in the areas of the endometriotic foci and adhesion scores compared to the rats in the control group.[5-7,21] However, in addition to the high costs, it carries the risk of osteoporosis when used for long periods of time. Additionally, GnRHa treatment produces several troublesome side effects because of its strong antiestrogenic effects, and relapse might occur after the cessation of treatment. Due to these reasons, alternative treatment options are being investigated.

Tibolone is a steroid molecule from the group of molecules that act as estrogenic activator regulator in selective tissues.[11] It has an estrogenic activity in the central nervous system, bone, and genital tissues, while it does not have such effects in the breast tissue and endometrium, so it is mainly used for the management of climacteric symptoms in postmenopausal women.[11,23] Tibolone in the endometrium is irreversibly converted to its Δ-4 isomer that binds to both progesterone and androgen receptors.[11] Tibolone and its Δ-4 isomer induce estrogen inactivating enzymes 17β-hydroxysteroid dehydrogenase and sulfotransferase, inhibit sulfatase and enhance locally the deactivation of biologically active estrogenic metabolites.[11,12] Numerous studies have shown that to achieve add-back treatment of endometriosis, the use of tibolone increase the bone mineral density, reduces anti-estrogenic side-effects, however does not affect the development of endometriosis foci.[24-26]

In the menopausal period, the use of tibolone has been shown to induce endometrial atrophy.[27] Ettinger et al., investigated the endometrial effects of tibolone in 3519 post-menopausal patients, and they emphasized the negligible effect of tibolone on endometrial proliferation formation in the first three years of its use.[28] On the other hand, literature reveals endometriosis development with tibolone used for postmenopausal hormone treatment in some cases.[29] To the best of our knowledge, the primary efficiency of tibolone in endometriosis treatment has not been studied to date in a rat endo-

TABLE 2: Comparison of delta values (posttreatment -pretreatment) of areas of endometriotic foci and adhesion scores among the three groups.

	Control (n= 8)	Leuprolid acetate (n= 8)	Tibolone (n= 8)	p value
Areas of endometriotic foci (mm^2)	8 (-3-30)	25.5 (3-54)	16.5 (9-30)	0.018*
Adhesion scores	1 (0-3)	2 (0-4)	1.5 (0-2)	0.683

*p<0.05 when comparison between control vs leuprolid acetate groups and control vs tibolon groups.

metriosis model. Therefore, our study might be considered as the first to investigate the relationship between tibolone and endometriosis in a rat model. In our study, tibolone caused a regression in the endometriotic foci in the early period as with GnRHa use. The areas of endometriotic foci were significantly reduced in the group of rats that received tibolone (Group-3) when compared to the rats in the control group (The mean areas of endometriotic foci were 33.1 mm^2 and 15.3 mm^2, respectively). The level of the regression in the endometriotic foci was similar to the one that obtained with GnRHa treatment. Additionally, after a 4-week treatment period, the adhesion scores of the rats were significantly smaller compared to the scores of the rats in the control group. The primary aim of this study was the determination and evaluation of macroscopic appearance and adhesions scores. However, the regression in the endometriotic foci was not histopathologically graded, which is a limitation of our study.

In conclusion, the preliminary information obtained in this study suggests that despite estrogenic effects, tibolone can be used as an alternative agent in the treatment of endometriosis due to its androgenic and progestagenic bioactive products. However, our findings need to be confirmed by further detailed studies with histopathologic correlations.

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