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Effect of combined therapy GnRH-a plus add-back tibolone on endocrine hormone levels and bone loss in endometriosis patients.

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Abstract

Objective: This study is to compare the effect of combined therapy gonadotropin-releasing hormone agonist (GnRH-a) plus add-back tibolone on endocrine hormone levels and bone loss of endometriosis patients.

Methods: Totally 100 endometriosis patients were enrolled. They were randomly divided into 1.25 mg tibolone group and 2.5 mg tibolone group, with 50 patients in each group. The levels of bone gla-protein (BGP) and endocrine hormone levels were measured before treatment and at 24 weeks of treatment. Bone mineral density (BMD) was measured before treatment, at 24 weeks of treatment, at 6 months and 12 months after drug withdrawal.

Results: In both 1.25 mg tibolone group and 2.5 mg tibolone group, the serum endocrine hormone levels of estradiol, follicle-stimulating hormone, and luteinizing hormone at 24 weeks of treatment were significantly lower than those before treatment ($P<0.05$). Compared with before treatment, BGP level was significantly higher at 24 weeks of treatment in both groups ($P<0.05$). BMD of lumbar vertebra and femoral intertrochanteric at 24 weeks of treatment, at 6 months and 12 months after drug withdrawal were lower than those before treatment, but without significant difference. Furthermore, there were no significant differences in endocrine hormone levels, BGP or BMD between the two groups at all the time points.

Conclusion: Combined therapy GnRH-a plus add-back tibolone can reduce bone loss and low dose tibolone can maintain bone density.

Keywords: Gonadotropin releasing hormone agonists (GnRH-a), Add-back therapy, Tibolone, Endometriosis.

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Introduction

Endometriosis is an estrogen-dependent and a common chronic gynecological disease. The incidence rate in women of childbearing age is about 10%, second only to the uterus fibroids [1]. Vercellini et al. [2] reported that if no medication was used after endometriosis surgery, the 2-year recurrence rate of endometriosis could reach up to 30.4% and with an annual increase rate of 10%, the 5-year recurrence rate could reach 36%-50%.

GnRH-a is an analog of the natural gonadotropin-releasing hormone (GnRH). It has a similar effect as that of GnRH. However, its half-life may be extended to 1-6 h and its binding affinity to the receptor increases 100-200 folds, compared with that of GnRH [3]. GnHR-a can inhibit ovarian function and effectively reduce estrogen levels [4]. It also can act directly on ectopic endometrium, reducing angiogenesis and inhibiting survival of ectopic endometrium [5,6]. Therefore, GnRH-a is commonly used for symptomatic treatment and prevention of endometriosis with good clinical efficacy. However, the side effects of GnRH-a (such as hot flashes, night sweats, dizziness, fatigue, vaginal dryness, loss of libido, mood changes and bone pain) limit its long-term use [7,8]. According to the report by Sagsveen et al. [9], the bone mineral density (BMD) of lumbar vertebra decreased by 3.2% after a 6-month usage of GnRH-a and by 6.3% after a 12-month usage. The treatment course of GnRH-a is limited to 6 months because of the side effect of bone loss [10].

Tibolone is related with the 19-methyltestosterone family and is a synthetic steroid hormone with estrogenic actions. Tibolone not only can facilitate the therapeutic effect of GnRH-a, slow bone loss, but also can reduce or alleviate the discomfort of the patient during treatment [11-13]. Combined therapy GnRHa plus tibolone is safe and effective to alleviate pain and prevent bone loss [14]. However, the recommended dose of tibolone is not consistent.

In this study, the effect of combined therapy GnRH-a plus add-back 1.25 mg tibolone was compared with that of combined therapy GnRH-a plus add-back 2.5 mg tibolone. The endocrine hormone levels, including estradiol (E2), follicle-stimulating

hormone (FSH), and luteinizing hormone (LH), and bone loss of endometriosis patients were measured.

Materials and Methods

Clinical data of patients

Patients with endometriosis were admitted for treatment in the First Affiliated Hospital of Xinjiang Medical University from Mar 2014 to Mar 2015. All patients were diagnosed according to medical records and clinical symptoms, and, the diagnosis was confirmed by postoperative pathology analysis. Totally 100 cases of endometriosis patients were enrolled in this prospective study according to the following inclusion criteria and exclusion criteria. The inclusion criteria were as follows: 1) patients aged 21.5 to 45 years old and with requirement of fertility; 2) patients underwent laparoscopic conservative surgery; 3) no usage of any hormone drugs within six months before surgery; 4) patients with normal liver and kidney function and without blood disease or thrombotic disease. The exclusion criteria were as follows: 1) patients with both endometriosis and uterine fibroids; 2) patients with a history of oral contraceptives at 6 months before surgery; 3) HBV and/or HCV carriers or infected patients; 4) patients with increased levels of alanine aminotransferase, aspartate aminotransferase, creatinine or urea nitrogen; 5) patients with thromboembolic disease. The basic demographic data of patients were listed in Table 1. There was no significant difference in the basic demographic data of patients. Prior written and informed consent were obtained from every patient and the study was approved by the ethics review board of Xinjiang Medical University.

Table 1. *Clinical data of patients.*

	1.25 mg tibolone group (n=50)	2.5 mg tibolone group (n=50)
Age (year)	35.62 ± 6.25	36.29 ± 6.27
Menstrual period (day)	5.67 ± 2.25	5.82 ± 1.55
Menstrual cycle (day)	30.66 ± 8.25	28.96 ± 7.63
Pregnancy times	1.78 ± 1.04	1.95 ± 1.15
Number of children born	1.56 ± 0.83	1.74 ± 0.75
Body mass index (kg/m2)	22.21 ± 3.11	23.14 ± 4.50

Patient grouping and treatments

Patients were numbered according to the admission order. Then, patients with odd number were assigned into 1.25 mg group and those with even number were assigned into the 2.5 mg group. Finally, 50 patients were included in each group. At 2 days after surgery, all patients received subcutaneous injection with 3.75 mg GnRHa (leuprolide acetate; Beijing Bo Ente Pharmaceutical Co., Ltd., Beijing, China) in the front wall of the abdomen. The injection was performed once every 28 days and for 6 times. For patients in 1.25 mg tibolone group, tibolone was orally taken (1.25 mg/times/day) at the second time of GnRHa injection. The calcium (Caltrate, Wyeth Pharmaceutical Co., Ltd. Shanghai, China) each tablet contains 1.5 g of calcium carbonate and vitamin D3 125 IU was also orally taken. For patients in 2.5 mg tibolone group, the use of GnRH-a and calcium was the same as that in 1.25 mg tibolone group. The dose of tibolone was 2.5 mg/times/day. The oral treatment with tibolone and calcium was stopped after the last time of GnRha injection. Patients were followed up for 12 months after drug withdrawal.

Sampling

Before treatment and at 24 weeks of treatment, peripheral venous blood (2 ml) was collected from each patient after 12 h of fasting. The levels of bone gla-protein (BGP) and endocrine hormone were measured before treatment and at 24 weeks of treatment. BMD was measured before treatment, at 24 weeks of treatment, at 6 months and 12 months after drug withdrawal.

- Measurement of endocrine hormone levels and BGP level
- Automatic microparticle radioimmunoassay system was used to detect serum endocrine hormone levels of E2, FSH, and LH. BGP level was measured with ELISA.

Measurement of BMD

BMD was measured using dual-energy X-ray absorptiometry (DEXA). The BMD of lumbar vertebra L1-L4, and femoral intertrochanteric was measured. The measurement accuracy was 1% and the repeated measurement error was <1%. BMD was shown as g/cm.

Statistical analysis

SPSS 19.0 software was used for data analysis. Data are expressed as mean ± standard deviation (SD). Student's t-test was used to compare the statistical differences. $P<0.05$ was considered as statistically significant.

Results

Changes of serum endocrine hormone and BGP levels

The serum endocrine hormone (including FSH, LH, and E2) and BGP levels were measured before treatment and at 24

weeks of treatment. As shown in Table 2, serum levels of FSH, LH and E2 at 24 weeks of treatment were all significantly lower than before treatment in 1.25 mg tibolone group (P<0.05). Moreover, serum BGP level was significantly increased at 24 weeks of treatment (P<0.05). Similarly, in 2.5 mg tibolone group, compared with those before treatment, FSH, LH and E2 levels at 24 weeks of treatment were also significantly decreased whereas BGP level at 24 weeks of treatment was significant increased (P<0.05) (Table 2). However, there was no significant difference in FSH, LH, E2 or BGP between the 1.25 mg tibolone group and the 2.5 mg tibolone group at 24 weeks of treatment (P>0.05) (Table 3).

Table 2. *Levels of FSH, LH, E2 and BGP in serum of 1.25 mg tibolone group and 2.5 mg tibolone group before treatment and at 24 weeks of treatment. Note: Before treatment vs. at 24 weeks of treatment in each group, *P<0.001.*

		Before treatment	At 24 weeks of treatment	t	*P
FSH (U/L)	1.25 mg tibolone group	12.43 ± 7.15	2.21 ± 1.98	9.741	<0.001
	2.5 mg tibolone group	12.64 ± 8.85	3.01 ± 2.32	7.443	<0.001
LH (U/L)	1.25 mg tibolone group	8.82 ± 2.67	3.62 ± 1.87	11.280	<0.001
	2.5 mg tibolone group	9.81 ± 6.32	4.35 ± 2.13	5.789	<0.001
E2 (pg/L)	1.25 mg tibolone group	65.76 ± 26.12	26.62 ± 8.37	10.090	<0.001
	2.5 mg tibolone group	66.08 ± 25.43	27.02 ± 6.83	10.489	<0.001
BGP (ug/L)	1.25 mg tibolone group	13.13 ± 2.48	16.84 ± 2.99	9.811	<0.001
	2.5 mg tibolone group	13.79 ± 2.63	17.12 ± 3.56	9.905	<0.001

Table 3. *Comparison between 1.25 tibolone group and 2.5 mg tibolone group at 24 weeks of treatment.*

	2.5 mg tibolone group	1.25 tibolone group	t	P
FSH (U/L)	3.01 ± 2.32	2.21 ± 1.98	1.855	0.067
LH (U/L)	4.35 ± 2.13	3.62 ± 1.87	1.821	0.072
E2 (pg/L)	27.02 ± 6.83	26.62 ± 8.37	0.262	0.794
BGP (ug/L)	17.12 ± 3.56	16.84 ± 2.99	0.483	0.665

Change of BMD level

To determine the effect of tibolone on BMD, the BMD level of the lumbar vertebra and femoral intertrochanteric was measured before treatment, at 24 weeks of treatment and at 6 months and 12 months after drug withdrawal. In 1.25 mg tibolone group, no significant difference was observed in BMD level of the lumbar vertebra and femoral intertrochanteric among all time points (P<0.05) (Table 4). The same case was found in 2.5 mg tibolone group, without any statistical significance among all time points (P<0.05) (Table 4). Furthermore, the differences in BMD level of the lumbar vertebra (Table 5) and femoral intertrochanteric (Table 6) between the two groups were not significant (P>0.05).

Table 4. *BMD level in 1.25 tibolone group and 2.5 tibolone group.*

Groups	Time point	Lumbar vertebra	t	P	Femoral intertrochanteric	t	P
1.25 mg tibolone group	Before treatment	1.092 ± 0.078	-	-	0.815 ± 0.067	-	-
	At 24 weeks of treatment	1.065 ± 0.073	1.79	0.08	0.798 ± 0.065	1.288	0.2
	At 6 months after drug withdrawal	1.075 ± 0.064	1.19	0.24	0.804 ± 0.064	0.84	0.4
	At 12 months after drug withdrawal	1.079 ± 0.071	0.87	0.39	0.806 ± 0.061	0.702	0.48
2.5 mg tibolone group	Before treatment	1.095 ± 0.073	-	-	0.810 ± 0.068	-	-
	At 24 weeks of treatment	1.078 ± 0.059	1.28	0.2	0.796 ± 0.067	1.037	0.3

At 6 months after drug withdrawal	1.081 ± 0.062	1.03	0.3	0.810 ± 0.058	0	1
At 12 months after drug withdrawal	1.085 ± 0.065	0.72	0.47	0.813 ± 0.062	-0.23	0.82

Table 5. *Comparison of BMD level of the lumbar vertebra between the 1.25 tibolone group and the 2.5 tibolone group.*

	2.5 mg tibolone group	1.25 tibolone group	t	P
Before treatment	1.095 ± 0.073	1.092 ± 0.078	0.199	0.843
At 24 weeks of treatment	1.078 ± 0.059	1.065 ± 0.073	0.979	0.330
At 6 months after drug withdrawal	1.081 ± 0.062	1.075 ± 0.064	0.476	0.635
At 12 months after drug withdrawal	1.085 ± 0.065	1.079 ± 0.071	0.441	0.660

Table 6. *Comparison of BMD level of the femoral intertrochanteric between the 1.25 tibolone group and the 2.5 tibolone group.*

	2.5 mg tibolone group	1.25 tibolone group	t	P
Before treatment	0.810 ± 0.068	0.815 ± 0.067	-0.370	0.712
At 24 weeks of treatment	0.796 ± 0.067	0.798 ± 0.065	-0.152	0.880
At 6 months after drug withdrawal	0.810 ± 0.058	0.804 ± 0.064	0.491	0.624
At 12 months after drug withdrawal	0.813 ± 0.062	0.806 ± 0.061	0.569	0.571

Discussion

The patients enrolled in this study were all diagnosed with endometriosis and underwent laparoscopic conservative surgery. The effects of combined therapy GnRH-a plus add-back tibolone on hormone levels and bone loss were investigated in these patients. Furthermore, the effects of 1.25 mg tibolone and 2.5 mg tibolone were compared. Our findings may shed light on reducing the adverse effects caused by GnRH-a in the combined therapy.

The combined therapy is based on the "estrogen threshold theory" put forward by Barbieri et al. [15], which suggests that if estrogen levels will be controlled within a low range (30-45 pg/ml), the maximum inhibition of ectopic endometrial growth and bone loss will be achieved. The inhibitory effect of the combined therapy GnRHa plus add-back estrogen on bone loss was also supported by the results reported by Olive DL et al. [16]. However, it is also reported that this combined therapy cannot completely prevent bone loss. For example, Pierce et al. [17] reported a 6-year follow-up on 49 cases of endometriosis patients and found that the BMD was not yet returned to normal bone density. In this study, we found that FSH, LH and E2 levels were significantly decreased whereas BGP level was significant increased at 24 weeks of treatment in both groups. Moreover, no significant difference was observed in BMD level of the lumbar vertebra and femoral intertrochanteric among all time points in both groups. Our results suggest that GnRH-a plus add-back tibolone treatment are effective in treating endometriosis.

The recommended dose of tibolone is 2.5 mg/d [18,19]. However, Xue et al. [20] reported that 2.5 mg/d tibolone caused endometrial hyperplasia and vaginal bleeding. Currently, the recommended dose of tibolone is 1.25 mg/d by the Endometriosis Collaborative Group of the Chinese Medical Association of Obstetrics and Gynecology [21]. Consistently, our results showed that there was no significant difference in hormone levels or BMD level between 1.25 mg tibolone group and 2.5 mg tibolone group. This indicates that 1.25 mg tibolone could also effectively improve the low estrogen symptoms and reduce bone loss. Thus, low dose of 1.25 mg tibolone can be used during add-back therapy.

There are some limitations in this study. First, the sample size was relatively small. Second, control group with GnRH-a treatment alone was not set up. In the future, studies with larger sample size and proper control group are warranted.

In summary, our findings demonstrate that combined therapy GnRH-a plus add-back tibolone can reduce bone loss and are as effective in treating endometriosis.

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Disclosures

All authors declare no financial competing interests.

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