

MENOPAUSE

Safety and efficacy of tibolone and menopausal transition: a randomized, double-blind placebo-controlled trial

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Objective: To evaluate the efficacy, safety and tolerability of Tibolone use during the menopausal transition (MT). **Methods:** Sixty-five healthy women aged 40–55 years (48.5 ± 3.5 years) were recruited for a randomized, double-blind controlled trial. Thirty participants were recruited to receive oral Tibolone 2.5 mg/day – Tibolone Group (TG), and 35 participants were assigned to the Placebo Group (PG), which received one capsule of lactose/day. Both groups were treated for 12 consecutive weeks. The Blatt-Kupperman Menopausal Index (KMI) and the Greene Climacteric Scale (GCS) were used. The glycaemic and lipid profiles, biochemical measures of hepatic function and endometrial thickness were measured for safety. A daily registry of complaints related to the treatment was maintained, and anthropometric measures were obtained to assess tolerability. **Results:** A total of 57 women completed the study. After 12 weeks of Tibolone use, the total score and percentage of the KMI and GCS were significantly decreased compared to baseline, which reflected the efficacy of the treatment of climacteric symptoms. The improvement in blood biochemistry, endometrial atrophy and maintenance of the anthropometrical measures reflected the safety of Tibolone use. The absence of serious side effects demonstrated good tolerability for Tibolone use. **Conclusions:** The results showed good efficacy, tolerability and safety of Tibolone use during the MT.

Keywords: Endometrium, hormone replacement therapy, menopausal transition, menopause, tibolone

Introduction

Variability in ovarian function occurs during the menopausal transition (MT) and results in the fluctuation of oestrogen concentration levels that are associated with a drop in progesterone and a progressive increase in follicle-stimulating hormone (FSH) levels [1–4]. Approximately 80% of MT women experience climacteric symptoms (CS), such as hot flashes, sweating, mood changes, decreased libido, vaginal dryness, and sexual avoidance, which affect the quality of life (QoL) and require treatment [3,5].

Hormone therapy (HT) with oestrogen alone or in combination with progesterone remains the gold standard for the treatment of climacteric syndrome [5,6]. Additionally, the use of several alternatives to HT have been proposed during the MT, such as

Tibolone, phytoestrogens and selective oestrogen receptor modulators (SERMs), with significant differences in patient adherence and safety and efficacy profiles [6–10].

Tibolone is a tissue-specific compound that is structurally related to 19-nortestosterone derivatives. It is as effective as HT for the relief of CS and the prevention of osteoporosis in postmenopausal women [9–13]. Two important positive effects are the improvements in sexual dysfunction and mood [9,10,12,13]. The risks are the same as HT but without endometrial stimulation and breast proliferation [9–11,13].

Tibolone has been used in Europe since 1988 by approximately 1.5 million women/year, but its use has been limited to postmenopausal women [9–11,13]. Because the associated risks of Tibolone use are similar to conventional HT, it is very important to address the efficacy and safety of Tibolone use in MT women using randomized clinical trials. Therefore, the aim of our study was to evaluate the efficacy of oral Tibolone (2.5 mg/day) on CS during the MT and assess its tolerability and safety profile using clinical and metabolic parameters.

Methods

Subjects and study design

A randomized, double-blind placebo-controlled study was performed in 65 Brazilian women in MT aged 40 to 55 years old (48.5 ± 3.5 years) to evaluate the efficacy, safety profile and tolerability of Tibolone use. To calculate the sample size, we estimated that 10% of the initial population (652 women enrolled in a specialized outpatient clinic for climacteric symptoms) would be sufficient to detect a 15% minimum difference between the groups with a power of 80% and a level of significance of $\alpha = 0.05$. The study protocol was approved by the Ethics Committee of the Federal University of Rio Grande do Norte (protocol number 294/08) and is registered under the Australian New Zealand Clinical Trials number 12611000200987. All the participants signed an informed consent form. The inclusion criteria included the following factors: (a) age between 40 and 55 years; (b) menstrual irregularity during the previous 6 months but fewer than 12 months of amenorrhea; (c) the presence of a uterus without anomalies in an initial vaginal ultrasonography evaluation and an endometrial thickness measurement ≤ 10 mm; (d) plasma FSH level ≥ 30 mUI/ml; (e)

blood biochemistry within normal limits {total cholesterol (Ct) < 250 mg/dl, triglycerides \leq 200 mg/dl and glycaemia \leq 100 mg/dl}; (f) a Blatt-Kupperman Menopausal Index (KMI) score \geq 14 points; and (g) mammography study that resulted in a BI-RADS (Breast Image Reporting and Data System method [14]) classification of 1 or 2. The following factors were the exclusion criteria: (a) current smoker; (b) use of any hormonal, psychotropic or other medications that could interfere with the lipid-glycaemic profile within 90 days of trial; (c) history of clinical hepatic or kidney disease or diabetes mellitus; (d) cerebrovascular, thyroid, cardiovascular or thromboembolic disorders; (e) any neoplastic disease; (f) hypertensive disorder (systolic BP \geq 160 mmHg and/or diastolic BP \geq 105 mmHg); (g) obesity [body mass index (BMI) > 30 kg/m²]; and (h) mammographic study that resulted in a BI-RADS [15] classification of a 3 or 4 [14,16–22]. The initial screening included a socio-demographic inventory; anamnesis for clinical and gynaecological concerns; and a detailed physical examination, including the measurement of blood pressure, waist and hip circumferences, weight, height and BMI. Pilot tests were conducted with 12 women to evaluate the applicability of the instruments that assessed the efficacy, tolerability and safety of Tibolone. We evaluated the climacteric symptomatology using the Greene Climacteric Scale (GCS [23]) and the KMI [24]; these instruments have been used previously in the Brazilian population [17–19]. GCS and KMI scores were determined at the onset of treatment and after 12 weeks. Blood samples were collected during the initial screening after a 12-h overnight fast to measure hormonal and biochemical parameters, including FSH, serum Ct and its fractions, triglycerides, glycaemia, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), albumin and globulin levels. Biochemical parameters were assessed at the onset of the study and after 12 weeks of treatment using an auto-analyzer Dimension AR[®] system (Dade Behring, Newark, NJ, USA) and the reagents and protocols from the manufacturer. Serum concentrations of oestradiol were determined during the follicular phase at baseline and after 12 weeks by radioimmunoassay using commercial kits from Diagnostic Products Corporation Kits, (Los Angeles, CA, USA). The Safety Committee was established consisting one gynaecologist and two pharmacists, who watched for adverse effects and could halt the trial if risks appeared. All women who met the inclusion criteria were randomly allocated into two groups (2 \times 2): the Tibolone Group (TG) received 2.5 mg/day of Tibolone (ReduClim[®], Farmaquímica, Brazil) orally in capsule form, and the Placebo Group (PG) received a daily administration of identical capsules that contained lactose. The ReduClin[®] tablets were encapsulated in Pharmatechniques Laboratory of the Federal University of Rio Grande do Norte for a member of the Safety Committee and were similar to the of placebo capsules. Other same member of the Safety Committee was responsible for the randomization and also assessed subject's compliance to the study protocol. Both groups received daily capsules for 12 weeks. Participants who failed to appear for at least one subsequent session or failed to follow the dosing regimen for more than 3 consecutive days within a 30-day period were considered non-compliant.

Endometrial safety and tolerability

Endometrial safety was evaluated by endometrial thickness using a model SSD-3500 ultrasound device (Aloka Co., Tokyo, Japan) that was attached to an endovaginal transducer at 5–9 MHz. Endometrial thickness (in mm) was determined as the distance

between one endometrium-myometrium interface and the opposite interface. The ultrasonography exams were performed by one investigator with a competence certificate and extensive experience with the method. Measurements were performed at baseline and after 12 weeks of treatment.

Tolerability to tibolone was evaluated using biochemical and anthropometric measures (BMI, waist-hip ratio and Waist circumference), and the occurrence of various side effects was investigated during home visits. Each participant received a diary notebook to record the dose ingested and the occurrence of side effects. The main predicted reactions were vaginal bleeding/spotting, pelvic pain, breast pain or tenderness [10,20], allergy, weight gain, headache, epigastralgia and androgenic effects [9,12]. A bleeding episode was defined based on the number of days of uninterrupted bleeding and the number of pads or tampons/day that were required [20]. Any clinical or laboratory changes resulted in the cessation of treatment by the Safety Committee.

Statistical analysis

Statistical analysis was performed using SPSS, version 15.0 for Windows. For the analysis within each group, we compared the results at the beginning and end of the study using Student's *t*-test for paired samples. For the analysis between treatment groups, Student's *t*-test for independent samples was used. To compare proportions between categorical variables, we used Pearson's χ^2 test (χ^2). An intention-to-treat analysis (ITT) was performed using all of the data from the allocated participants in each group. Throughout the analysis, a *p* value of 0.05 and a confidence interval of 95% were considered significant.

Results

Baseline characteristics

Overall, 57 of the 65 selected women (87.7%) completed the study. In the TG, 3/30 women (10%) discontinued the trial due to vaginal bleeding, a lack of study adherence or a change in address. In the PG, 5/35 women (14.2%) discontinued the trial due to a weight increase, a lack of study adherence and/or aggravation of the CS. There were no significant differences in the baseline demographic and clinical characteristics between the two groups (Table I).

Efficacy, safety and tolerability

Table II shows the average of the total KMI and GCS scores according to groups. A considerable decrease in climacteric symptomatology was observed in the TG compared to the control group as measured with both scores. Vasomotor symptomatology decreased in the TG to 92% of the basal score.

Table III shows the analysis of the effects of Tibolone on lipid and biochemical parameters, endometrial thickness and weight measurements between baseline and 12-weeks values in both groups. After 12 weeks of treatment, the TG showed a significant reduction in the levels of Ct 12.8% low density lipoprotein cholesterol (LDLc) 10%, high density lipoprotein cholesterol (HDLc) 14%, triglycerides 36%, glycaemia 6% and the triglycerides/HDLc ratio 25.7%, compared to their baseline averages. The plasma levels of estradiol as well as other variables of interest did not differ significantly between the basal measurements and after 12 weeks of treatment in either group. The endometrial thickness was reduced to 1.6 mm (21%) in the TG compared to the basal average (*p* < 0.001), which is consistent with endometrial atrophy after 12 weeks. The other parameters were not significantly different. The analysis of adverse

Table I. Basal characteristics of the participants.

Variables	Tibolone (n=30)	Placebo (n=35)	p
Age (years)	48.0±3.6	49.0±3.6	0.24
Age at menarche (years)	12.3±1.4	12.5±1.6	0.28
Educational level (years)	7.8±4.3	7.5±3.8	0.78
BMI (kg/m ²)	26.2±0.6	26.0±0.6	0.84
Waist circumference (cm)	91.8±1.4	91.7±1.4	0.93
Waist-to-hip ratio	0.79±0.1	0.81±0.1	0.79
Endometrial thickness (mm)	8.1±2.6	7.9±3.4	0.44
Systolic blood pressure (mmHg)	122.7±2.5	124.7±2.4	0.55
Diastolic blood pressure (mmHg)	77.7±1.3	77.2±1.2	0.79
FSH basal (mIU/ml)	75.0±23.2	70.7±26.1	0.48
Oestradiol (pg/ml)	62.3±13.4	61.9±12.8	0.77
KMI basal (score total)	35.5±6.7	36.0±10.1	0.82
Greene score total	36±3.3	36.7±3.7	0.76
Total cholesterol (mg/dl)	220.0±38.8	223.2±38.9	0.80
HDLc (mg/dl)	42.2±9.3	46.8±10.9	0.83
LDLc (mg/dl)	144.0±36.9	142.5±35.9	0.87
Triglycerides (mg/dl)	152.2±53.0	177.6±90.7	0.20
Triglycerides/HDLc ratio	3.6±0.9	3.8±1.1	0.77
Glucose (mg/dl)	90.5±6.7	90.9±9.7	0.81
Total protein (mg/dl)	7.3±0.56	7.4±0.38	0.89

Data are presented as the means and S.D.

Table II. Mean scores of the Greene Climacteric Scale and Blatt-Kupperman Index (KMI) before (Baseline) and after treatment for 12 weeks with Tibolone or placebo.

Variables	Baseline	Tibolone group	Placebo group
KMI	35.5±8.4	11.3±6.5*	28.6±11.5
Greene total score	31.9±4.6	19.8±3.5*	38.0±4.6
Greene psychological cluster	17.1±7.3	11.0±6.5*	17.9±6.8
Greene anxiety subcluster	10.0±4.3	6.4±4.0*	10.7±3.4
Greene depression subcluster	7.1±3.0	4.6±3.1*	7.4±4.1
Greene somatic cluster	12.1±4.3	7.4±3.2*	12.8±4.7
Greene vasomotor cluster	5.0±1.3	0.4±1.0*	4.9±1.6
Greene sexual interest	2.2±1.0	1.4±0.8*	2.3±0.7

Average values ± S.D. *p < 0.001 vs. baseline and placebo groups.

symptoms as interpreted by the participants showed that the treatment was well tolerated in the TG. However, vaginal bleeding/spotting and breast pain/tenderness were registered within the first four weeks of treatment in 12.3 and 30.7% of participants, respectively.

Discussion

The results of the present study support that Tibolone is an effective alternative for the treatment of CS during the MT because relevant reductions in both the KMI (68.2%) and GCS (36.5%) scores were observed after a 12-week period of Tibolone use. Because the MT is associated with deterioration in sexual life, mood changes and a significant impairment in the QoL of these women, Tibolone emerges as an available therapeutic option for MT women [9,10,16].

Tibolone exerts a global oestrogenic action through 3 α and 3 β -OH-tibolone, which are connectors and activators of oestrogen receptors [9,25,26]. The ability of these metabolites to modulate peripheral α -oestrogenic receptors is the

Table III. Effects of tibolone on lipid and biochemical parameters, endometrial thickness and weight measurements between baseline and 12-week values in both groups.

Variables	Baseline	Tibolone group	Placebo group
Total cholesterol (mg/dl)	221.9±39.3	193.3±37.9*	223.9±30.7
HDL (mg/dl)	46.4±10.1	39.6±7.2	42.5±6.3
LDL (mg/dl)	143.2±36.4	129.0±43.6*	150.2±26.2
Triglycerides (mg/dl) ratio	164.9±71.9	105.2±26.8*	155.5±55.0
Triglycerides/HDLc	3.6±1.1	2.7±0.8*	3.7±0.9
AST (U/L)	25.9±7.6	28.4±6.3	25.4±5.4
ALT (U/L)	37.9±16.5	34.7±16.6	38.3±10.7
GGT (U/L)	39.2±28.9	30.5±16.5	36.0±24.9
Glucose (mg/dL)	90.7±8.2	85.6±8.0*	91.4±7.7
Total protein (mg/dL)	7.3±0.5	7.1±0.4	7.3±0.5
Albumin (mg/dL)	4.2±0.4	4.4±0.5	4.3±0.6
Globulin (mg/dL)	3.2±0.5	2.7±0.5	3.0±0.6
Endometrial thickness (mm)	8.1±2.6	6.4±1.9*	7.9±2.4
Oestradiol (pg/ml)	62.1±13.1	63.9±12.2	65.8±11.6
BMI (kg/m ²)	25.9±3.5	26.3±3.8	25.9±3.7
Waist circumference (cm)	91.1±8.1	90.6±7.8	91.0±9.0
Waist-to-hip ratio	0.8±0.1	0.8±0.1	0.8±0.1

Average values ± S.D. *p < 0.001 vs. baseline and placebo groups. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ -glutamyl transpeptidase.

basis for the decrease in climacteric symptomatology that has been reported in placebo-controlled clinical experiments [16,25,26].

The efficacy of Tibolone therapy for the relief of vasomotor symptoms is similar to oestrogen use [9,10]. Somunkiran et al. [16] have corroborated this result in a randomized clinical experiment in women in MT who were 47.8±3.2 years old.

Our results in somatic, sexual and psychological symptoms, as evaluated with the GCS, were similar to the results by Somunkiran et al. [16]. The action of Tibolone on somatic and psychological symptoms is due to its positive neuroendocrine effects on the modulation of opioid and serotonergic receptors, the increase in plasma and pituitary β -endorphin levels, and the stimulated production of allopregnanolone, a steroid with sedative and anxiolytic properties [9,10,16,26].

In previous studies on Tibolone use, headache was reduced in the TG (85.2% p=0.05). This result is consistent with a study by Lam et al. [27], in which tibolone was compared with placebo. Moreover, Somunkiran et al. [16] compared Tibolone and 17 β -oestradiol and found that headache only occurred in the Tibolone group.

Our study also showed that the positive effects of tibolone on psychological symptoms were similar to the effects observed by Somunkiran et al. [16]. However, the results of Lam et al. [27] were inconclusive because Tibolone-treated and placebo-treated participants showed the same improvement in psychological well-being as evaluated by GCS scores.

The favourable action of tibolone on sexual interest in the present study is due to its oestrogenic and androgenic actions [10,27]. The oestrogenic action, which occurs via the 3 α and 3 β -OH-tibolone metabolites, relieved vaginal dryness and dyspareunia, which increases comfort during sexual activity. The androgenic action, which is exerted by the parent Tibolone molecule and its derived isomeric Δ^4 , reduces plasma levels of sex hormone-binding globulin (SHBG) and increases the concentration of free testosterone, DHEA-S and oestradiol, which increases sexual desire [10,26,27].

Lipid metabolism during the MT fluctuates due to biological ageing and the hormonal changes that occur during this period [11,28]. As a consequence, increases in Ct, triglycerides and LDLc occur, but HDLc levels remain unchanged [9,10,28]. The action of Tibolone on the lipid profile is complex but shows favourable, protective effects against atherosclerosis in both animal models and clinical, placebo-controlled experiments [9,10,12,28].

In Tibolone-treated patients, triglycerides showed the greatest reduction relative to their basal values (36.2%), followed by Ct (12.8%), LDLc (10%) and HDLc (14.6%). These results are similar to the clinical experiments reviewed by Nicoletta et al. [10]. The reduction in HDLc is due to the stimulation of hepatic lipases by the Δ^4 isomer [11–13].

The OPAL study (Osteoporosis Prevention and Arterial effects of tiboLone [26]) found that the use of Tibolone during menopause did not increase the risk of atherosclerosis compared to equine oestrogen and medroxyprogesterone. This result is partially explained by experimental studies in animal models that found that Tibolone preserved the ability of HDLc to remove free cholesterol particles from peripheral tissues [29]. Kloosterboer et al. [30] demonstrated that Tibolone reduced HDLc levels in the first 12 months of therapy. However, the values returned to pre-treatment levels after 36 months. Stuckey et al. [29] demonstrated that the use of fenofibrate with Tibolone did not correct the alterations in HDLc levels and suggested that Tibolone might exert unknown effects on the vascular system.

In our study, the participants in the TG showed a reduction of approximately 6% in their fasting glycaemia after 12 weeks of treatment. These results are consistent with Crona et al. [31], who observed a reduction in the tolerance to glucose in young oophorectomised women who used Tibolone. Freitas et al. [19] administered Tibolone at 2.5 mg/day for 6 months to 24 postmenopausal women with non-insulin-dependent diabetes mellitus and found no change in fasting glycaemia during treatment. In addition, Wiegatz et al. [32] conducted a multi-centre study of postmenopausal women and compared the effects of Tibolone with the effects of conjugated oestrogens on carbohydrate metabolism; the authors found no significant clinical differences between pre- and post-treatment in either group.

In our study, we observed a 25.7% reduction in the triglyceride/HDLc ratio in the TG. This ratio is a powerful predictor of insulin resistance and the risk of coronary artery disease [29]. Therefore, we infer that the use of Tibolone during the MT may provide effective and longer cardioprotection, as reported by other studies [9,10,16,28,29,31].

The biotransformation of Tibolone occurs in the liver and intestines, and its steroid metabolites undergo complete hepatic aromatization by p450 complex enzymes [13]. Despite the possibility of hepatotoxicity, the use of Tibolone for 12 weeks did not cause either an increase in transaminases or altered liver function, which is consistent with previous studies [10,16,25,30,31,33].

The beginning of HT treatment is associated with irregular uterine bleeding and, which are attributed to excessive oestrogen activity [6,34]. In our study, the TG subjects experienced vaginal bleeding/spotting and breast symptoms that was restricted to the first 4 weeks of treatment, which is similar to Landgren et al. [25]. According to Meuwissen [34], this bleeding only occurs in women during MT or early post-menopause with detectable oestrogen levels.

After 12 weeks of treatment, there was a 21% reduction in endometrial thickness compared to baseline thickness. This reduction was due to the tissue-selective action of Tibolone,

which acts through the Δ^4 metabolite isomer in the human endometrial cells to link the β -progesterone receptors and protect the uterus from the oestrogenic effects of Tibolone [10,25,33].

The duration of our study (12 weeks), the daily dose used (2.5 mg of tibolone), the scales that were used to evaluate the climacteric symptoms and the participant profiles were all defined according to previous clinical experiments [9,10,27,33,34]. The use of two different daily doses of tibolone (1.25 and 2.5 mg) in a multi-centre randomized, double-blind placebo-controlled study showed similar effectiveness in the treatment of moderate and severe vasomotor symptoms and in genital atrophy, which suggests that the efficacy of tibolone in smaller doses [25]. Therefore, it is possible that a daily tibolone dose of 1.25 mg during MT could produce similar results without the discomfort of the initial adverse effects.

Conclusions

The results of our study suggest that Tibolone is effective for the reduction of vasomotor, sexual, psychological, and somatic symptoms in women during MT. Tibolone also improved glycaemic and lipid profiles and showed good tolerance and clinical and endometrial safety. Nevertheless, additional experiments are needed to better define how effective Tibolone and its metabolites would be at other doses and treatment durations.

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