EFFECTS OF TIBOLONE ON MARKERS OF BONE METABOLIC ACTIVITY IN POSTMENOPAUSAL WOMEN

UTICAJ TIBOLONA NA MARKERE METABOLIČKE AKTIVNOSTI KOSTI KOD ŽENA U POSTMENOPAUZI

Snežana Marić Krejović[1], Aleksandar Živanović[2], Sandra Živanović[3], Rade Marković[4]

[1]Health Center Užice – Health Care Institution Arilje, Serbia
[2]Faculty of Medicine, University of Kragujevac, Serbia
[3]Health Center Kragujevac, Serbia
[4]General Hospital Užice, Serbia

Summary: Osteoporosis, a systemic disease of the bones, is a serious health and socio-economic problem because of its consequences, i.e. broken bones. It is believed that 10% of the world's population suffers from osteoporosis and it affects mostly postmenopausal women (postmenopausal osteoporosis). Tibolone is a synthetic steroid that has estrogenic, androgenic, and progestagenic properties. It has been used primarily for the prevention of postmenopausal osteoporosis and treatment of climacteric symptoms. The research included a group of 40 postmenopausal women with osteopenia treated with tibolone. The control group included 40 postmenopausal women who were not taking any medication. Control group patients were older (54.5 ± 9.84) than the patients treated with tibolone (51.6 ± 6.22). Bone metabolic activity was evaluated using osteocalcin (N-MID osteocalcin) for bone formation and CTX I for bone resorption. Blood samples were taken before therapy was introduced and 3 months after its introduction. The average value of osteocalcin after three months of tibolone therapy was 26.32 ± 3.312 ng/mL compared to the average osteocalcin value prior to therapy of 29.6 ± 3.343 ng/mL. The average value of CTX I three months after tibolone therapy of 0.2870 ± 0.0783 ng/mL was lower compared to the average CTX I value before the therapy of 0.4539 ± 0.1144 ng/mL. Our results show the efficacy of tibolone in preventing bone loss, which was highly statistically significant. They also reveal its suppressive effects on bone formation and resorption, but these effects are statistically less significant. Tibolone signif-

Kratak sadržaj: Osteoporoza, sistemsko oboljenje kostiju, predstavlja ozbiljan zdravstveni i socijalno-ekonomski problem zbog svojih posledica, preloma kostiju. Smatra se da 10% svetske populacije boluje od osteoporoze, a posebno su ugrožene žene u postmenopauzi. Tibolon je tkivno specifičan steroid sa estrogenim, progestagenim i androgenim svojstvima. Prvenstveno se koristi za prevenciju postmenopauzalne osteoporoze i ublažavanje klimakteričnih tegoba. Istraživanje je sprovedeno u grupi od 40 žena sa osteopenijom u postmenopauzi tretiranih tibolonom. Kontrolnu grupu činilo je 40 žena u postmenopauzi koje nisu uzimale nikakvu terapiju. Pacijentkinje kontrolne grupe bile su starije (54,5 ± 9,84) od pacijentkinja tretiranih tibolonom (51,6 ± 6,22). Od parametara metaboličke aktivnosti kosti korišćeni su osteokalcin kao parametar formiranja kosti i CTX I kao parametar resorpcije kosti. Krv je vađena pre uvođenja terapije i 3 meseca nakon uvođenja terapije. Prosečna vrednost osteokalcina tri meseca nakon terapije tibolonom je bila niža (26,32 ± 3,312 ng/mL) u odnosu na prosečnu vrednost osteokalcina pre terapije (29,6 ± 3,343 ng/mL). Prosečna vrednost CTX I tri meseca nakon terapije tibolonom je bila niža (0,2870 ± 0,0783 ng/mL) u odnosu na prosečna vrednost CTX I pre terapije (0,4539 ± 0,1144 ng/mL). Naši rezultati pokazali su izrazito koristan uticaj tibolona na koštanu resorpciju i supresivni efekat na koštano formiranje, što proističe iz povezanosti između formiranja i resorpcije kosti. Tibolon izrazito smanjuje nivo koštane resorpcije kod pacijentkinja u postmenopauzi sa osteopenijom. Njegov uticaj na formiranje kosti je

Address for correspondence:

Dr Snežana Marić Krejović
Health Center Arilje
Women's Health Care Service
Str. Vojvode Stepe 8. 31 230 Arilje
+38131/896-501; +38163/10-46-573
e-mail: snezana.krejovic@gmail.com

List of abbreviations: BMD, bone mineral density; OC (N – MID osteocalcin) – serum osteocalcin; b-CTx (b-CrossLaps, CTX I) – serum C terminal cross-linked telopeptide of type I collagen; ECLIA, electroluminescent immunochemistry method; DXA, dual energy X-ray absorbtiometry; WHO, World Health Organization; IOF, International Foundation for Osteoporosis and Bone Diseases.

icantly reduces the level of bone resorption in post-menopausal women with osteopenia. Its effects on bone formation are less expressed. The parameters of bone meta-bolic activity are a very useful diagnostic means in the evaluation of tibolone effects on bone metabolic activity and in the prognosis of bone mass loss.

Keywords: osteoporosis, tibolone, postmenopause

manje izražen. Praćenje parametara metaboličke aktivnosti kosti veoma je korisno dijagnostičko sredstvo u proceni efekta tibolona na metaboličku aktivnost kosti i eventualno u prognozi konačnog ishoda za koštanu masu.

Ključne reči: osteoporoza, tibolon, postmenopauza

Introduction

Osteoporosis, as a systemic disease of the bones, represents a serious health and socio-economic problem due to its consequences – bone fractures. It is believed that 10% of the world's population suffers from osteoporosis and postmenopausal women are especially at risk. The risk of fracture caused by osteoporosis in women aged 50 is 40%, while in men of the same age it is 13% (1, 2). Osteoporosis is a generalized bone disease characterized by abnormal bone strength increases causing a predisposition to fractures. Bone strength involves the bone quantity (bone mass-density, size of the bone) and quality (microarchitecture, mineralization of the matrix, bone metabolism, accumulated microscopic damages, damages of the trabecular and cortical bone) (3). Early diagnosis of osteoporosis is possible only by measuring bone mineral density (BMD – Bone Mineral Density), and the recommended method is based on the application of low energy X-rays (dual energy X-ray absorbtiometry – DXA), which measure bone density in vertebral bodies and femoral neck. A normal result for bone mineral density is not more than one standard deviation (SD) lower than the mean value of maximum bone mass in young, healthy women aged between 20 and 30 (T score).

Osteopenia indicates the measured bone mineral density, which is 1 to 2.5 SD (10–25%) less than the maximum bone mass in young, healthy women aged between 20 and 30 years (T score). Osteoporosis means bone density that is more than 2.5 SD (25%) lower than the maximum bone mass in young, healthy women. These are the screening diagnostic criteria set and accepted by the World Health Organization (WHO), International Foundation for Osteoporosis and Bone Diseases (IOF) and the Foundation for Osteoporosis of the USA (4).

The goal of therapy in osteoporosis is to prevent fractures and the consequences of fractures, and to maintain mobility and quality of life. In recent years, more so than in the past, hormone replacement therapy in postmenopausal women has included the preparations of testosterone. In postmenopausal women the concentration of testosterone is reduced by about 50%, while this reduction is even greater in women with initial adnexectomy. The ovaries in postmenopausal women continue to produce testosterone due to increased stimulation of stromal cells, owing to high concentrations of gonadotropins. During the first

three years of menopause, it is considered that the concentrations of androstenedione, testosterone, dihydrotestosterone, and dehydroepiandrosterone sulfate are still stable (5). Tibolone is a tissue-specific steroid with estrogenic, progestagenic and androgenic properties. As a synthetic steroid hormone it has a structural formula very similar to the 19-norethisterone and norethynodrel. After oral use the tablets are quickly absorbed and metabolized into three compounds that have the pharmacological effects of tibolone preparations. Two metabolites (3-α OH tibolone and 3-β-OH tibolone) predominantly express estrogen activity that is reflected in the mitigation of climacterium symptoms, and effects on the bones, vagina and blood vessel walls. A third metabolite (Δ4 isomer of tibolone) has progestagen and androgen activity, exhibiting its influence on the endometrium, the effect of antiestrogens in the breasts and the walls of veins and mild androgenic effects on mood and libido.

Clinical trials have shown that tibolone prevents bone loss and maintains skeletal integrity in postmenopausal women (6, 7). One study examined the three-year effect of tibolone at a dose of 2.5 mg daily on bone mineral density. After the first year of this therapy there was a highly significant increase in bone mineral density, after the second year it was continued, and after the third year the difference was significant (8). The authors suggest that this therapy can be used by women getting along in years, who have osteoporosis and do not want to experience vaginal bleeding. Bone biomarkers and markers of bone changes are the indicators of bone metabolism derived from the bone matrix or bone cells. In making a diagnosis of osteoporosis, in addition to medical history, assessment of the risk factors, clinical examination and certain laboratory tests, the most important is the determination of mass and bone density and the assessment of bone metabolic activity (8, 9). The dynamic state of bone metabolism could also provide information about early pathological changes in the bones and the risk of some diseases of the locomotor apparatus. By measuring the concentrations of bone markers information can be obtained quickly on the therapeutic response in relation to the measurement of bone density. Significant changes in the value of bone markers can be detected soon, three months after the introduction of therapy, while the change in bone density can be evaluated only after one year of therapy introduction (10, 11). In this paper osteocal-

cin and CTX I are used as biochemical markers of bone turnover.

The USA National Committee for Clinical Laboratory Standards (NCCLS, USA, www.nccls.org) published in 2004 the guidelines for the use of bone biomarkers.

Recommendations for the use of bone markers:

– in identifying individuals with increased bone metabolism

– for predicting the risk of bone fractures among women after menopause

– in assessing the response to treatment in patients with osteoporosis or risk of osteoporosis when the treatment can be carried out with antiresorptive or anabolic agents.

The aim of the study is to determine whether tibolone effects the parameters of bone metabolic activity in postmenopausal women with decreased bone density.

Material and Methods

Fourty postmenopausal patients with decreased bone density (osteopenia) who participated in this prospective, controlled clinical study were treated with tibolone (Livial ®) at a dose of 2.5 mg per day. The study also included 40 postmenopausal patients (control group) with decreased bone density (osteopenia) who were not treated with any kind of therapy.

The criteria for including patients in the study were:

1. Natural or surgical (hysterectomy) postmenopausal women (one year after the last menstrual period to five years after menopause);

2. Low bone mineral density (T <-1) confirmed by the results of densitometry (DXA).

In this study we have used two bone markers, one as a marker of bone resorption (CTX I) and the other as a marker of bone changes (N-MID osteocalcin). Since bone markers can very quickly give information about the success of therapy, after three months of starting therapy in all female patients the serum values of bone markers were reestimated. At the repeated blood sampling and determination of bone biomarkers, three months after therapy initiation, blood sampling was performed under the same conditions, as the levels of bone markers are to some extent influenced by circadian rhythm.

Venous blood samples for the determination of serum bone markers were taken in the morning (from 7.00 to 8.00 h) after a night of refraining from eating and before therapy. Immediately after it was taken, the blood was centrifuged, separating the serum, and frozen at –20 °C until the analysis. Prior to the freezing, the samples were checked for hemolysis,

since erythrocyte proteases dissolve osteocalcin, thereby affecting the level of β-CTx (manufacturer's notice). After the whole sample was collected, the serum was analyzed for specific bone markers. The level of the most stable mid-fragment of osteocalcin (N-MID osteocalcin) as the marker of bone formation was determined, as well as the β-CrossLaps (CTX I – bone resorption marker) via the »ECLIA« (electrochemiluminescence immunoassay) on an Elecsys 2010 automated analyzer (Roche Diagnostics GmbH, Germany). Reference values for serum CTX I: premenopausal women 0.016–0.573 ng/mL, postmenopausal females 0.016–1.008 ng/mL.

Reference values for serum osteocalcin: premenopausal women 11–43 ng/mL, postmenopausal females 15–46 ng/mL, women with osteoporosis 13–48 ng/mL.

Statistical methods

In the statistical analysis standard methods of descriptive statistics have been used. Apart from these multivariate statistical methods, the analysis of variance for two- and multi-factor analysis of outcomes and results of the treatment have been done in order to determine the best treatment protocol and recommendations for remediation of osteopenia.

Results

Control group patients were older (54.5 ± 9.84) than the patients treated with tibolone (51.6 ± 6.22), but this difference was not statistically significant (p = 0.20). Thus, the two studied groups were comparable with respect to age. In all female patients osteocalcin and CTX I were determined before and three months after therapy. Descriptive statistics for osteocalcin and CTX I before treatment in these two groups are shown in *Table I*.

The *Table I* shows that the average value of osteocalcin before therapy in the patients treated with tibolone was higher than in the control group, which had a much lower average value of osteocalcin. By analyzing these data with the ANOVA test, a statistically significant difference was determined between the groups for the average of osteocalcin before therapy (p = 0.000).

Table I Parameters of bone turnover before therapy.

Group	N	Osteocalcin ng/mL $\bar{x} \pm SD$	CTX I ng/mL $\bar{x} \pm SD$
Tibolone	40	29.6±3.343	0.4539±0.1144
Control group	40	17.84±4.003	0.2550±0.4997

Table II Parameters of bone turnover in the course of therapy.

Group	N	Osteocalcin ng/mL $\bar{x} \pm SD$	CTX I ng/mL $\bar{x} \pm SD$
Tibolone	40	26.32±−3.312	0.2870±0.0783
Control group	40	18.98±3.495	0.2620±0.0448

Not only the markers of bone formation were monitored, but also the bone resorption marker CTX I. The *Table II* shows that the average value of CTX I before therapy in the patients treated with tibolone is higher than in the control group, which has a much lower average value of CTX I. By analyzing the data with the ANOVA test it was established that there is a statistically significant difference between the groups with respect to the average value of osteocalcin before therapy (p = 0.000). The osteocalcin and CTX I levels were then analysed three months after the treatment, as well as in the control group that did not use any treatment. Descriptive statistics of osteocalcin and CTX I within the groups are shown in *Table II*.

The *Table II* shows that the serum level of osteocalcin after the application of tibolone was significantly lower compared with the pre-treatment level (p = 0.01). Serum level of osteocalcin in the control group not taking the treatment was slightly and statistically insignificantly higher than three months before. ANOVA test revealed that there is a statistically significant difference between the groups in the average of osteocalcin after three months (p = 0.000).

The table shows that the serum level of CTX I in the tibolone treated group is significantly lower compared to the pre-treatment level (p = 0.000). Serum level of CTX I in the control group that did not take treatment was slightly and statistically insignificantly higher than in the period three months before. By analyzing these data with the ANOVA test, it was determined that there is a statistically significant difference between the groups in the average value of osteocalcin after three months (p=0.000).

Discussion

This paper presents the processed results of the clinical application of tibolone at a dose of 2.5 mg per day orally in the treatment of women with osteoporosis in postmenopause. For the evaluation of the treatment effect the determination of two parameters of bone metabolic activity was done, one of which shows the degree of bone formation (osteocalcin), and the other one the level of bone resorption (CTX I). Of key interest to our research was the monitoring of these parameters during the therapy and comparison of the obtained results with the pre-treatment levels. Our results have shown a significant effect of tibolone on bone resorption, which is highly statistically significant. This effect, according to our results, was evident after 3 months of starting therapy. This fact is very important for clinicians because by monitoring this metabolic parameter the final desired therapeutic effect can be predicted very quickly in terms of increasing bone mass. Our results have shown a suppressive effect on bone formation, which certainly resulted from the coupling of formation to resorption, but this effect was less statistically significant.

In this study, two bone markers, as the indicators of the activity of osteoblasts and osteoclasts during bone remodeling, were monitored: osteocalcin, as a marker of bone formation, and CTX I, as a marker of bone resorption. In the results, the values of the control group are presented, but because the markers themselves are exclusively used as indicators of the therapeutic effects, this group is shown only for comparison with the tibolone group. Many studies indicate that if the therapy has effect, a decline in osteocalcin should be expected. The effect of tibolone on postmenopausal women indicates that three-month therapy with this drug reduces the concentration of serum osteocalcin by 50% (12). Of course, these are the women without osteoporosis, so the effect of therapy is significantly higher in such a short period of time. The eight-year follow-up of the women who were treated with tibolone and the control group shows that the average values of osteocalcin vary, but without significant variation. However, the values of osteocalcin were lower in the women who received tibolone than in the control group. The reason for this is a small change in BMD in women who were on tibolone treatment, while within the control group of women constant fall in BMD was recorded (13). From the results of this study we can conclude that the tibolone treatment is effective even in healthy women and has a relatively protective effect on the bones. Results of our study regarding the effect of tibolone on osteocalcin are consistent with the results of other researchers, namely there is a positive effect of tibolone on bone metabolism in women with osteopenia in the postmenopausal period. Since, as stated previously, the control group is used for comparison only, we can conclude that after three months osteocalcin is not significantly different in the control group, just as the osteocalcin at the beginning of therapy.

Besides osteocalcin, the patients were monitored for CTX I, as a marker of bone resorption. Specifically, bone metabolism is reflected in the balance of synthesis and absorption, so it is logical that within the analysis of bone metabolism both processes are observed together. Successful treatment includes increased synthesis and decreased bone resorption. If

the treatment is effective, it is expected that osteocalcin and CTX I will fall, that is, the values of the two markers in the blood will be reduced. By analyzing the literature data on tibolone and CTX I the first thing we can conclude is that the number of researchers who have monitored this bone marker is far smaller than of the researchers who have used other markers. Furthermore, some of the researchers who used the same marker analyzed it in the urine and not in serum. However, a certain number of researchers monitored this marker in serum. According to a study of Delmas et al. (14) on the effects of raloxifene on bone mineral density in postmenopausal women with osteopenia, there is a significant difference in the average values of this marker over time.

Specifically, this study analyzed the changes in the value of this marker for two years, and based on the results it was established that the change in the average CTX value in the first trimester after introduction of therapy is important, and that it is 22% of the initial value. In our study the decrease from the initial value is 37%, which is far more than in the group of patients from the study of Delmas et al. (13). The difference between our study and the study of the author named lies primarily in the fact that in the mentioned study the women were older and on average in postmenopause for 17 years, which resulted in a major fall in the T score compared to our patients. Comparison of other characteristics of our patients and the ones from the study of Delmas et al. (14) revealed that the patients did not differ significantly regarding other characteristics. According to this study, the decrease in CTX I is the highest in the sixth and twelfth month, but since our study relates to a shorter term, we are unable to compare these values at these two points in time. However, what we can conclude by comparing our results with the results of this study is that tibolone has a major effect on the change in CTX I, and that since it is about women in early menopause, this rate of decline of the bone marker in our study is higher than in the study of Delmas et al. (14). Tibolone therapy, as a form of antiresorptive treatment, distinctly reduces the level of bone resorption in postmenopausal women with osteopenia, while its effect on bone formation is less pronounced. This effect of tibolone may be considered a favorable metabolic milieu for increasing bone mass and thus reducing the risk of fracture. The effect of tibolone on bone metabolism is evident by 3 months after the therapy introduction. Monitoring the parameters of bone metabolic activity such as osteocalcin and CTX I is a very useful diagnostic means in evaluating the effect of tibolone on bone metabolic activity, and eventually in the prognosis of final outcome concerning bone mass. Also, individually observed, CTX I is of greater value.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

References

1. Kanis JA, Johnell O, Oden A, et al. Long-term risk of osteoporotic fracture in Malmo. Osteoporosis International 2000; 11: 669–74.

2. Cooper G. Epidemiology of osteoporosis. Osteoporosis International 1988; 8 Supl 3: 1.

3. Osteoporosis prevention, diagnosis and therapy. NIH consensus statement 2000; 17: 9.

4. Utian WH. Health beyond menopause. Satellite Symposium at IOF World Congress on Osteoporosis. Rio de Janeiro, 2004; 4–6.

5. Longcope C, Franz C, Morello C, et al. Steroid and Gonadotropin Levels in Women During the Perimenopausal Years. Maturitas 1986; 8: 189–96.

6. Modelska K, Cummings S. Tibolone for post-menopausal women: systematic review of randomized trials. J Clin Endocrinol Metab 2002; 87: 16–23.

7. Moore RA. Livial: A review of clinical studies. Br J Obstet Gynecol 1999; 106 suppl 19: 1–21.

8. Ettinger PM. Aging Bone and Osteoporosis. Archives of Internal Medicine 2003; 163: 2237–46.

9. Ponorac N, Rašeta N, Radovanović D, Matavulj A, Popadić-Gaćeša J. Bone metabolism markers in sportswomen with menstrual cycle dysfunctions. Journal of Medical Biochemistry 2011; 30: 135–140.

10. Engler H, Koeberle D, Thuellmann B, Senn HJ, Riesen WF. Diagnostic and prognostic value of biochemical markers in malignant bone disease: A prospective study on the effect of biphosphonate on pain intensity and progression of malignant bone disease. Clin Chem Lab Med 1998; 36: 879–85.

11. Demers LM. Clinical usefulness of markers of bone resorption and formation. Scand J Clin Lab Invest 1997; 227: 12–20.

12. Frenkci V, Yilmazer M, Frenkci S. Effects of short-time (3 months) tibolone treatment on bone turnover in postmenopausal women. Arch Gynecol Obstet 2003; 268 (2): 85–7.

13. Rymer J, Robinson J, Fogelman I. Effects of 8 years of treatment with tibolone 2.5 mg daily on postmenopausal bone loss. Osteoporosis International 2001; 12 (6): 478–83.

14. Delmas PD, Davis SR, Hensen J, Adami S, Nijland EA. Effects of tibolone and raloxifene on bone mineral density in osteopenic postmenopausal women. Osteoporosis International 2008; 19 (8): 1153–60.