Original article

Mechanism of Bazedoxifene/conjugated estrogens drugs therapy and its Clinical effect on osteoporosis

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Abstract. *Objective:* To observe the clinical effects and explore the mechanism of Bazedoxifene/conjugated estrogens upon bone targeting in the treatment of postmenopausal osteoporosis *Methods:* The study group was inclusive of 211 postmenopausal osteoporotic patients who were under treatment with Bazedoxifene/conjugated estrogens drugs in the study hospital during the period January and December 2018. The control group contained 56 patients and were treated only with calcium. The researchers analyzed the risks involved in the adverse events such as bone mineral density, osteoprotegerin (OPG) and insulin-like growth factor (IGF) and fracture prior to and after the treatment. *Results:* The study results inferred that the clinical treatment effective rate was 88.39% in Study group whereas it was 23.21% in the control group and were statistically significant ($P<0.05$). Prior to treatment, the researchers identified no significant difference in mean lumbar positive position (L2-4) and right femoral neck bone density IL-1β in between the groups ($P>0.05$). However, after the treatment, the study group data showed high mean lumbar positive position (L2-4) and heavy right femoral neck bone density in comparison with the control group ($P<0.05$). When compared to the control group ($P<0.05$), the Study group experienced less number of adverse events after 12 months of treatment. *Conclusion:* Bazedoxifene/conjugated estrogen is proved in the current study as an efficient drug to treat postmenopausal osteoporosis. It has the ability to arrest the rapid loss of bone mass, enhance the bone density, mitigate fracture risks and effectively reduce the symptoms of menopause.

Keywords: Estrogen; Bazedoxifene; Osteoporosis; Clinical effect

Introduction

Osteoporosis is a bone disease which is caused either by synthesis of too much bone or loss of too much or sometimes both. The bones of osteoporotic patients become weak and break from a fall. In few serious cases, minor bumps also occur [1]. Being a bone disease, the characteristics of this disease are destruction of the microstructure of bone and reduction in the bone mass while the former results in enhanced bone brittleness. A number of people exhibit no symptoms till the time they met with a bone fracture. Osteoporosis can be treated via healthy diet, medication and weight-bearing exercise which altogether aim at prevention of further bone loss and/or strengthen the existing weak bones [3]. Osteoporosis proliferation and the absence of estrogen are directly associated, especially at the time of perimenopause and menopause.

Bone mass may get reduced during specific periods such as early menopause (before 45 years) and in case of any prolonged periods. This might be attributed to the secretion of low amount of hormones accompanied by absent or irregular menstrual periods [4].

The most common type of osteoporosis reported so far is postmenopausal osteoporosis which occurs due to estrogen deficiency. When estrogen is deficient, it leads to high level of bone turnover which in turn impact all kinds of bone cells. As almost all the women undergo estrogen deficiency during post-menopause period, estrogen replacement has been found to be one of the important therapeutic measures for such patients [5] [6]. Anti-osteoporotic effect is exerted by estrogen through cell receptor pathways, when it is supplemented exogenously as per the studies conducted earlier. The estrogen receptors perform directly at osteoblast and osteoclast surface [7-8].

In this pathway, the first step would be the varied actions i.e., ER-agonist/antagonist activity, of Selective Estrogen Receptor Modulators (SERMs), a class of compounds based on the target organs. The postmenopausal osteoporosis is prevented as well as treated with the help of SERMs. Having been established as a third-generation selective estrogen receptor modulator, Bazedoxifene (BAZEDOXIFENE/CONJUGATED ESTROGENS) is still reported for its clinical effects and relative mechanisms in the treatment of postmenopausal osteoporosis patients. The current study critically evaluates the evidence on the usage of **Bazedoxifene/conjugated estrogens** (a third-generation SERM), in combination with conjugated estrogens (CE), among postmenopausal women.

Materials and Methods

General information

The study hospital approved the current study to be conducted and the study period was between June 2017 and December 2019. The patients (n=211) who were diagnosed with postmenopausal osteoporosis and under treatment with Bazedoxifene/conjugated estrogens were considered as the Study group.

Selection criteria

The inclusion criteria of the study were as follows: (1) patients who met the diagnostic criteria for osteoporosis [9] i.e., T value ≤-2.5; (2) postmenopausal women whose time of menopause should've been ≥2 years (3) reported primary back pain, peripheral bone pain and knee hip pain symptoms and (4) patients who agreed and signed for the study with informed consent and cooperated for the follow-up. The exclusion criteria of the study were as follows (1) secondary osteoporosis (2) patients diagnosed with impairment of kidney, liver or cardiac functions (3) diagnosed with tumor or other metabolic diseases and (4) who were undergoing immunosuppressive agents, glucocorticoids for a long-time. A total of 56 patients who were treated only with calcium was placed under the control group.

The treatment regime for control group was calcium erqi D3 tablets (wyeth pharmaceutical co., LTD and each tablet contained vitamin D3 125U^{+} calcium carbonate 0.6g. One tablet was orally taken by the patients twice a day. In case of Study group, they were treated with calcium hormone therapy in addition to erqi D3 tablets (manufacturer: wyeth pharmaceutical co., LTD., national drug approval (H10950029) i.e., One tablet was orally taken by the patients twice a day. The researchers selected Viviant Tablets (BAZEDOXIFENE ACETATE) for the hormone therapy (manufacturer: Pfizer; Specification: 20 mg/tablet, 100 tablets/box). One table was taken orally only once per day. Doctors framed the dietary guidance for patients under treatment with a focus on nutritive values: calcium-rich foods such as tofu, small high calcium milk, dried small shrimps, seaweed, kelp, etc., Further, few other exercises were included for the patients to follow such as increasing their light time, 1 hour compulsory physical activity such as (jogging, gymnastics, tai chi exercise and do less the trunk flexion, rotation and other movements), complete restriction of smoking and limit alcohol consumption.

Observation indicators

Prior to and after the treatment, various factors such as tumor necrosis factor (TNF-α),

osteoprotegerin (OPG), risk of bone mineral density (BMD), insulin-like growth factor (IGF), Visual Analogue Scale (VAS) and other adverse events were observed. VAS scores tend to be sensitive towards the non-pharmacological as well as pharmacological procedures which change the experiences of pain. It also has a high correlation with pain measured using verbal and numerical rating scales. VAS scores can be utilized to determine the degree of pain among the patients prior to and after 12 months of treatment. This pain coverage includes anterior flexion, roll over pain, lumbago back pain and posterior extension pain. When the score is high, it automatically denotes heavy pain intensity. When post-surgical patients (hysterectomy or laparoscopic myomectomy or knee replacement) were checked for their post-operative pain intensity, based on the distribution of pain, using VAS scores, the scale values are no pain (0–4 mm), mild pain (5–44 mm), moderate pain (45–74 mm), and severe pain (75– 100 mm) (**11**) with no values available for normative values.

Bone mineral density test which is otherwise called as Dual energy X-ray absorptiometry (DXA, Madison, USA) makes use of X-rays to determine the quantity of minerals. Prior to and after the treatment, this test was done to calculate the lumbar orthosis (l2-4) and bone mineral density (BMD) of the right femoral neck of patients in both groups.

Biochemical estimation

The manufacturer's instructions were followed to estimate the serum osteoprotegerin using ELH-OPG-1 human-OPG-ELISA kit [RayBiotech Inc., Norcross, USA]. In this analysis, 100 µl standard or sample was added to each well and then kept under incubation for 2 hours and 30 minutes at room temperature or else overnight at 40°C. Each well was then added with 100 µl prepared biotin antibody. At room temperature, again this was incubated for about one hour and 100 µl streptavidin solution was added. In the next step, 45 minutes of incubation was maintained followed by washing step i.e., final one. The remaining conjugate was permitted to undergo reaction with the substrate H_2O_2-tetramethylbenzidine (TMB). Each well was then added with 100 µl TMB one-step substrate reagent. Acid solution was added so as to prevent from the occurrence of further reactions. The resultant yellow product' absorbance was measured at 450 nm. Anti-insulin or anti-IGF-1 antibody was utilized by the assay system for performing phase immobilization while the study used another anti-insulin or IGF-1 antibody in the antibody–enzyme (horseradish peroxidase) conjugate solution as well.

In case of the presence of IGF-1 or human insulin in the sample, it tends to react with antibody on the well and enzyme conjugate, thus producing sandwiched structures of insulin or IGF-1 molecules with solid phase and enzyme-linked antibodies in each side. Tetramethylbenzidine solution was added to this and kept under incubation which finally ended up in blue color formation. The insulin or IGF-1 concentration and test sample's color intensity are directly proportional to each other.

Serum interleukin-1β (IL-1β) IL-1β assay: By following the guidelines from the manufacturer, human IL-1βIL-1β was determined with highly sensitive ELISA kits (procured from R&D Diagnostics, Wiesbaden, Germany) which are specific for human cytokines. Cytokine assays in case of every patient and his/her matched control were run in the same lot. In order to perform this test, the blood sample (5mL venous blood) was collected the patients with empty stomach. The sample was centrifuged at 3,500 rpm for 10 minutes and serum was collected. The serum was refrigerated at -20°C for inspection

Statistical methods

The study used SPSS 20.0 statistical software whereas the counting data was expressed by rate (n%). The authors used χ^2 test. The measurement data were expressed as mean ± standard deviation ($\bar{x}$±s). When the two mean values were compared between the groups using t test, $P < 0.05$ indicated a statistically significant difference.

Results

Comparison of treatment effect between the two groups

There was no statistically significant difference in age, body mass index, disease course and other general information between the two groups (P > 0.05) (**Supplementary table 1**), indicating comparability. The study found no statistically significant difference as shown in the table 1 between the groups in terms of disease course, body mass index, age and other such general information thus it denotes the comparability (P > 0.05). The study group had a total effective rate of 88.39% while it was 23.24% in control group. There was a statistically significant difference found between the groups (P < 0.05) as shown in the **table 1**. However, in terms of mean lumbar orthosis (l2-4) and bone mineral density of the right femoral neck between the groups, prior to the treatment, there was no statistically significant difference found (P > 0.05). As shown in **table 2**, after treatment, there was a statistically significant difference (P < 0.05) found and higher average lumbar orthosis (l2-4) and right femoral neck bone mineral density were observed in the study group compared to control group.

Comparison of fractures and other adverse events after treatment between the two groups

The Study group recorded significantly low number of incidence of fractures, spinal deformation, fatigue and other adverse events than the control group after

Supplementary Table 1. General data of study participants between the two groups

Group	Age	Body mass index (kg/m²)	Course of disease (year)
Study group (n=211)	42~71 (59.52±7.56)	18.52~27.12 (22.51±2.32)	1~5.3 (2.18±1.04)
Control (n=65)	44~69 (61.36±3.46)	18.71~25.01 (22.1±2.8)	1~5.1(2.15±1.21)
t	2.109	0.315	1.145
P	0.299	0.873	0.283

Table 1. Treatment effect between the two groups and their comparison [n (%)]

Group	Excellent	Good	Average	Poof	Total effective rate
Study group (n=211)	48(51.78)	41(36.61)	09(9.82)	1(1.79)	99,9(92.39)
Control (n=65)	13(15.35)	12(17.86)	19(30.36)	32(46.43)	21(25.11)
$\chi 2$					81.371
P					<0.005

Table 2. Bone mineral density and **L**umbar orthosis (l2-4) of the right femoral neck between groups

Group (g/cm^2, $\bar{X}$±s)	Lumbar orthosis (L2-4)		Right femoral neck	
	Before	After	Before	After
Observation(n=112)	0.75±0.09	0.77±0.06	0.65±0.06	0.84±0.17
Control(n=56)	0.85±0.06	0.63±0.07	0.65±0.11	0.81±0.03
t	2.054	15.742	0.882	14.111

12 months of treatment with statistically significant differences (P < 0.05). Both the groups exhibited no statistically significant difference in the occurrence of hot flashes and venous thromboembolism (P > 0.05).

Comparison of serum levels of OPG, IGF-1 and IL-1βbetween the two groups before and after treatment

When compared between the groups prior to treatment, no statistically significant difference found in the average levels of OPG, *IGF-1* and Serum interleukin-1β (IL-1β) (P > 0.05). However, there was an increase found in the study group in terms of average OPG and IGF-1 levels compared to the control group after treatment. The level of Serum interleukin-1β (IL-1β) was found to be less in the control group with statistically significant differences (P < 0.05) as shown in the table 3.

Discussion

During post-menopause period, ovaries stop producing estrogen while the fat tissues only produce the estrogens then. This results in creating a deficiency for estrogen [10]. Estrogen is an essential component in bone development and mandatorily required for appropriate closure of epiphyseal growth plates in females as well as males. In young skeleton, if the estrogen is deficient, it may result in osteoclast formation and increased levels of bone resorption. At the time of menopause, when estrogen becomes deficient, it triggers cancellous and cortical bone loss [11]. The bone metabolism in women is highly impacted by estrogen deficiency after the menopause [12]. A number of studies conducted earlier mentioned that Menopause Hormone Therapy (MHT) helps the body to maintain or enhance the BMD (Bone Mineral Density), mitigate the risks of osteoporotive fracture and prevents postmenopausal osteoporosis. But there is a wide range of treatment regimens available with different formulations, dosages, timings and durations with different therapies for individual characteristics of the patient, as these factors exhibit different role in the effect of MHT. When estrogen is supplemented exogenously i.e., hormone therapy with Bazedoxifene/conjugated estrogens, it can enhance the estrogen levels in the body. This results in returning to normalcy in bone metabolism and accordingly improved bone mineral density to some extent [13]. However, there has been negligible or no reports published at all with regards to therapeutic effect of Bazedoxifene/conjugated estrogens, the next-gen SERMs, upon postmenopausal osteoporosis patients. The current study examined the clinical impact of Bazedoxifene/conjugated estrogens in treating postmenopausal osteoporosis. The study analyzed the impact after 12 months of treatment with Bazedoxifene/conjugated estrogens. The results exhibited that there was a significant increase in the mean lumbar orthosis (l2-4), bone density of the right femoral neck and the total clinical response rate among the Study group containing postmenopausal osteoporosis patients compared to the calcium treatment (P < 0.05). This infers that the Bazedoxifene/

Table 3. Serum OPG, IGF-1 and Serum interleukin-1β levels between groups comparison before and after treatment (±s)

Group	OPG(pmol/L)		IGF-1 (μg/L)		TNF-α(pg/ml)	
	Before	After	Before	After	Before	After
Observation(n=112)	4.85±0.42	8.28±1.33	150.38±28.71	291.62±23.95	19.83±3.12	15.19±3.17
Control(n=56)	4.16±0.45	4.37±0.53	154.11±20.51	206.35±20.68	14.98±2.11	16.21±2.31
t	0.642	27.473	0.983	15.584	1.735	13.750
P	0.760	<0.005	0.272	<0.005	0.101	<0.005

conjugated estrogens were able to successfully improve the symptoms and bone density among the patients.

Further, there was a significant increase observed in the levels of OPG and igf-1 in the study group compared to the control group. However, the level of IL-1β was found to be low than the control group ($P < 0.05$). Both TNF as well as IL1 are able to trigger wide range of cell types to synthesize IL6 due to which it act as 'broad spectrum' cytokine by itself. Tumor necrosis factor (TNF-α) receptor family has numerous receptors among which OPG is one of the recently-added members and is otherwise termed as osteoclast inhibitor [14].

The osteoblasts are the primary secretors of OPG and it binds with nuclear factor B receptor activating factor ligand (RANKL) in order to hinder the signaling pathway of osteoclasts. In this way, it inhibits the damage of osteoclasts in bone [15]. As per the studies conducted recently [16], the formation as well as activation of osteoclasts get indirectly inhibited by OPG when it binds with OPGL. This way, OPG plays an important role as anti-osteoporotic factor. The study group exhibited notable enhancements in OPG as well as bone mineral density after receiving treatment with selective estrogen receptor modulator Bazedoxifene/Conjugated Estrogens. This phenomenon denotes that Bazedoxifene/Conjugated Estrogens prevented the creation and activation of osteoclasts through OPG enhancement. This way, it strongly inhibited osteoclasts' absorption to bone, resulting in increase in bone mineral density.

Being a polypeptide protein, the molecular structure of IGF is similar to insulin and is otherwise termed as growth promoting factor. IGF-1 is able to boost bone matrix synthesis, ensure bone mass balance, hinder the loss of calcium from bone and prevent bone catabolism [17-18]. The current study observed that IGF-1 got significantly upregulated after treating with Bazedoxifene / Conjugated Estrogens. This phenomenon infers that the Bazedoxifene / Conjugated Estrogens are able to promote IGF-1 secretion in vivo. While this scenario can promote osteoblast proliferation and effectively prevent as well as treat osteoporosis. The studies conducted earlier mentioned that the osteoblast differentiation gets inhibited by IL-1β [19] and when the latter increases, it can boost the apoptosis of osteoblasts [20].

When the patients were treated with Bazedoxifene/Conjugated Estrogens, there was a notable decrease found in the levels of IL-1β. This phenomenon infers that Bazedoxifene/Conjugated Estrogens has the potential to decrease the TNF-α levels. When it get decreased, the apoptosis rate of osteoblasts also gets reduced while the differentiation of osteoblasts becomes normalized. In this way, osteoporosis can be prevent and treated in an effective manner. Limitations of the study include sample size as it is considered as the most important parameter. Though the optimal sample size can be calculated, it is the availability of economic and human resources and time that decide the number of observations that can be carried out for the study.

Conclusion

To summarize, Bazedoxifene/Conjugated Estrogens is an effective drug that can be used to treat postmenopausal osteoporosis. It has the ability to prevent the rapid loss of bone mass, improve bone density, provide relief from osteoporosis back pain, systemic bone pain and other symptoms and also reduce the risks of fracture.

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