Pharmacology, Biochemistry and Behavior 129 (2015) 65–71

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Effects of chronic sodium alendronate on depression and anxiety in a menopausal experimental model

Rita Citraro [1], Luca Gallelli [1], Antonio Leo, Pasquale De Fazio, Patrizia Gallelli, Emilio Russo *, Giovambattista De Sarro

Science of Health Department, University of Catanzaro, Catanzaro 88100, Italy

ARTICLE INFO

Article history:
Received 12 August 2014
Received in revised form 10 December 2014
Accepted 17 December 2014
Available online 24 December 2014

Keywords:
Osteoporosis
Mood disorders
Anxiety
Alendronate
Menopause

ABSTRACT

Objective: During menopause, lower levels of estrogen may induce bone resorption as well as anxiety and depression. Bisphosphonates represent the first choice in the treatment of osteoporosis and no data are available concerning their effects on comorbid behavior alterations. Therefore, in this study, we evaluated the effects of chronic alendronate (1 mg/kg/day) on depression and anxiety in an experimental animal model of menopause.
Methods: Female Wistar rats were ovariectomized or sham operated at 6–7 months of age. Two weeks after surgery, rats were randomized into four treatment (24 consecutive weeks) groups: (1) vehicle-treated SHAM group, (2) alendronate-treated SHAM group, (3) vehicle-treated ovariectomized group, and (4) alendronate-treated ovariectomized group. After treatment, we evaluated both depressive- and anxiety-like behavior through forced swimming test (FST) and open-field test (OF). Finally, the inverted screen test was used to assess the incapacitating effects of ovariectomy in rats.
Results: We documented a significant and time-related increase in immobility times and in anxiety-like behavior in rats with ovariectomy in comparison to control sham group. Alendronate at 3 months, but not at 6 months, significantly decreased both immobility time and anxiety levels, but it significantly increased motor performance. Using the Pearson's test, we documented a significant correlation between behavior and motor performance.
Conclusion: Despite the apparent effects of alendronate on animal behavior, in our experiments, such effects seem to be mediated by an increase in motor performance.

1. Introduction

Osteoporosis is a serious worldwide health problem mainly affecting middle-aged and elderly women. It is characterized by both reduced bone mass and deterioration of bone microarchitecture leading to bone fragility and increased risk of fractures (Cummings and Melton, 2002).

Osteoporosis is a chronic disease of compromised bone strength that affects postmenopausal women. Since 1960, the link between menopause and osteoporosis was suggested; postmenopausal osteoporosis is a silent disease, which is often diagnosed until fractures occur with no previous symptoms. It is characterized by low bone mineral density (BMD) and changes in bone microarchitecture that reduce bone strength and increase fracture risk (Cummings and Melton, 2002). The cellular changes that occur in estrogen deficiency are now quite well understood (Tella and Gallagher, 2014). Estrogen deficiency is the major contributing factor to bone loss after menopause; therefore, estrogen treatment is considered the standard drug therapy for preventing bone loss and risk of fractures (Khan et al., 2014; Tella and Gallagher, 2014).

Commonly, bone resorption in menopausal women is related to reduced levels of estrogens (Lerner, 2006). Estrogen receptors, ERα and ERβ, are located in many brain areas and are also involved in the regulation of mood and behavior as well as in the pathophysiology of mood disorders (Halbreich and Kahn, 2001). Several studies have found an increased incidence of depression and anxiety in women across the menopausal transition (Woods et al., 2002). A clinical study performed in perimenopausal women with major mood disorders documented that estradiol treatment (patch 0.1–0.2 mg) combined with fluoxetine treatment (10–20 mg/day) decreases the severity of depression more effectively than fluoxetine therapy alone (Westlund Tam and Parry, 2003). Animal models confirmed an enhanced effect of fluoxetine treatment following estradiol administration (Recamier-Carballo et al., 2012). Moreover, it has been reported that hormonal contraceptive therapy in reproductive-age women is related with lower levels of depression, as well as decreased depressive disorders in women with major depression (Keyes et al., 2013).

* Corresponding author at: Science of Health Department, School of Medicine, University of Catanzaro, Viale Europa, Germaneto, Catanzaro 88100, Italy. Tel.: +39 0961 3694191; fax: +39 0961 3694192.
E-mail addresses: citraro@unicz.it (R. Citraro), gallelli@unicz.it (L. Gallelli), antonioleo78@alice.it (A. Leo), defazio@unicz.it (P. De Fazio), ncosta@virgilio.it (P. Gallelli), erusso@unicz.it (E. Russo), desarro@unicz.it (G. De Sarro).
[1] Equally contributed to the article.

http://dx.doi.org/10.1016/j.pbb.2014.12.006
0091-3057/

Estrogens are highly lipophilic hormones; therefore, they cross the blood–brain barrier, enter, and bind to intracellular estrogen receptors distributed in many of the areas related to anxiety and depression (Ostlund et al., 2003) such as the following: (1) raphe nucleus—both ERα and ERβ can be found in this area; ERβ appears to be the predominating ER subtype identified on serotonergic neurons in several species (Gundlah et al., 2001; Merchenthaler et al., 2004), and ERα may only be expressed in murine serotonergic cells (Sheng et al., 2004). Raphe contains postsynaptic 5-HT1a receptors (Burnet et al., 1995) that act as autoreceptors and inhibit serotonin release (Savitz et al., 2009). Experimental studies performed in animal models documented that estrogen treatment administered to ovariectomized rats and at mid-follicular levels to rhesus macaques decreases the expression of 5- HT1a autoreceptor (Henderson and Bethea, 2008; Le Saux and Di Paolo, 2005) and increases serotonin levels (Di Paolo et al., 1983). (2) Hippocampus: in this area that is strongly related to depression, 5-HT1a receptors are expressed postsynaptically, which receive projections from raphe neurons (Hornung, 2003) and increase serotonin release (Castro et al., 2003). In ovariectomized rats, estradiol treatment, probably via ERβ activation, decreases 5-HT1a receptor and consequently serotonin levels (Pandaranandaka et al., 2006). Moreover, it has been suggested that 5-HT1a receptor activation stimulates serotonergic neurons in the median raphe nucleus, producing an anxiogenic effect (Dos Santos et al., 2008). The hippocampus is susceptible to E2 decline and replacement in OVX rats. The role of E2 has been demonstrated in hippocampus-mediated affective behavior; chronic estradiol replacement into aged female rats reduces anxiety and depressive-like behavior also enhancing cognitive performance (Walf et al., 2009).

Another antidepressant mechanism of estrogens could be related with their neurotrophic and neuroprotective effects within the hippocampus (Scharfman and MacLusky, 2006). In particular, it has been documented that the administration of estradiol in ovariectomized rats induces an increase in the release of brain-derived neurotrophic factor (BDNF) and of vascular endothelial growth factor (VEGF),decreasing depressive-like behavior (Licht et al., 2011; Warner-Schmidt and Duman, 2007).

Estrogen represents the most effective compound in decreasing bone loss and improving mood disorders; however, its use is limited due to its carcinogenic risk and other related side effects (Pike et al., 2007). Further pharmacological osteoporosis treatments include calcium and vitamin D supplementation and drugs acting on bone tissue such as anti-resorptives (i.e., SERMs, hormonal replacement therapy, bisphosphonates, and denosumab), bone formers (teriparatide), and mixed agents (strontium ranelate) (Bhutani and Gupta, 2013).

Selective estrogen receptor modulators (SERMs; Raloxifene, Lasofoxifene, and Bazedoxifene) have evolved through multiple generations for the prevention and/or treatment of postmenopausal osteoporosis. SERMs act similarly to estrogens on bones and may be an appropriate option for women who cannot tolerate bisphosphonates. The combination of SERMs and estrogens may be an option for women at increased risk of fracture who are still experiencing vasomotor symptoms. Bisphosphonates, strontium ranelate, selective estrogen receptor modulators, denosumab, and teriparatide may improve the quality of bone reducing the risk of fracture in osteoporosis (Stevenson et al., 2005). Bisphosphonate (alendronate, risedronate, ibandronate, and zoledronate) and selective estrogen receptor modulators (e.g., bazedoxifene) represent the first choice for postmenopausal osteoporosis treatment, with demonstrated efficacy in reducing the incidence of vertebral fracture. Bisphosphonates have also shown significant improvements in BMD of the spine and hip. In contrast, denosumab and zoledronic acid are both potent antiresorptive drugs considered as an alternative for those patients who do not tolerate oral bisphosphonates (Lems and den Heijer, 2013; Maeda and Lazaretti-Castro, 2014). It was previously demonstrated that bisphosphonate dominates the conservation of bone trabecular geometry and structural strength, whereas strontium ranelate influences bone volume and material composition locally (Wu et al., 2013). Therefore, bisphosphonates are the most used antiresorptive agents for the treatment of osteoporosis. New therapies for osteoporosis include oral calcitonin, sclerostin inhibitors, integrin antagonists, and cathepsin-K inhibitors (Maeda and Lazaretti-Castro, 2014).

Bisphosphonates, although indicated for the treatment and prevention of bone health disturbances such as osteoporosis, can cross the blood–brain barrier; in fact, they were found to be potent inhibitors of cholesterol biosynthesis from mevalonate mimicking the action of lipophilic statins. The levels of total brain cholesterol were shown to correlate positively with the amount of amyloid beta (Aβ), which is characteristic for Alzheimer disease. Therefore, bisphosphonates were also indicated as a possible treatment for AD prevention and/or treatment (Cibickova et al., 2009). Furthermore, bisphosphonates could have central-behavioral effects through a modulation of neurosteroid synthesis. Neurosteroids are potent and effective neuromodulators that are synthesized from cholesterol in the brain. Increasing evidence indicates that dysregulation of neurosteroids production plays a role in the pathophysiology of stress and stress-related psychiatric disorders, including mood and anxiety disorders (Zorumski et al., 2013).

Ovariectomy (OVX), change of diet, drugs, immobilization, breeding, central control of bone mass are commonly used as osteoporosis animal model. The ovariectomized rat model is most commonly used in research on postmenopausal osteoporosis. After ovariectomy, bone resorption exceeds bone formation causing bone loss. Soon thereafter, bone remodeling reaches a steady state, where resorption and formation are balanced (Behr et al., 2012; Kalu, 1991; Lelovas et al., 2008). To date, no data have been published concerning the effects of bisphosphonate on mood disorders. Therefore, in this study, we aimed at the evaluation of the effects of alendronate (ALE) on depressive- and anxiety-like behavior in an ovariectomized female rat model of menopause.

2. Materials and methods

2.1. Animals

Female Wistar albino rats, of 6–7 months of age and a body weight of 250–300 g, were purchased from Charles River Laboratories s.r.l. (Calco, Lecco, Italia). Following arrival, animals were housed three/four per cage and kept in room controlled for both temperature and humidity (22 ± 2 °C, 60 ± 5%) and with a reversed light/dark (12/12 h) cycle (light on at 19:00 h) at least for one week before surgery. All rats were given free access to food and water until the time of experiments. Procedures involving animals and their care were conducted in conformity with the international and national law and policies (European Communities Council Directive of 24th November 1986, 86/609EEC). All efforts were made to minimize animal suffering and to reduce the number of animal used.

2.2. Experimental protocol and drug administration

After 2 weeks of acclimatization, 20 female Wistar rats underwent bilateral OVX to induce osteoporosis and 20 were sham operated, followed the same surgical procedure with the exception of ovaries removal.

On estrus day, all rats were anesthetized with a mixture of tiletamine/zolazepam (1:1; Zoletil 100®; 50 mg/kg i.p.; VIRBAC Srl, Milan, Italy) and then placed supine. Ovariectomy was performed via a midline abdominal incision (2 cm in length) in the linea alba. The ovarian ligaments and cervix were ligated with 5–0 silk, using a single-clamp technique. The ovaries and the uterus were then