AN EXPERIMENTAL STUDY EVALUATING THE INFLUENCE OF BISPHOSPHONATES ON DEPRESSION PATTERNS IN SWISS ALBINO MICE AND WISTER RATS

AMITHA N, S. S. TORGAL*

Department of Pharmacology and Pharmacotherapeutics, J. N. Medical College, Belagavi 590010, Karnataka, India
Email: ss.torgal.hm@gmail.com

ABSTRACT

Objective: Bisphosphonates are used for treating osteoporosis. Few studies have reported their effect on alterations in comorbid behaviour such as depression. Therefore, present study was performed to investigate the effects of bisphosphonate drugs on depression in adult male Wister rats and Swiss albino mice.

Methods: The study was conducted on adult male Wister rats and Swiss albino mice, 36 of each type, equally divided into six groups. One group was classified as control group and the rest were treated as test groups. Initial photoperiod of 12 h was provided for acclimatization, prior to the start of the experiment. Drug administration was not performed in control group. Forced swimming test and tail suspension test were performed to investigate the antidepressant activity. Locomotor activity was performed to evaluate the action of drugs on the nervous system. Effects of the test drugs were compared with a standard drug-amitriptyline. Results were statistically evaluated by one-way analysis of variance followed by Bonferroni's multiple comparison test. $P \leq 0.05$ was considered significant.

Results: In forced swimming test, duration of immobility was significantly reduced in the standard and test drugs when compared to control group; however, it was not significant in all the four test groups as compared to that of amitriptyline-administered group ($p > 0.05$). In tail suspension test, significant decrease ($p < 0.01$) in the duration of immobility was observed with administration of drugs when compared to control group. Results of test groups were not found to be significant as compared to amitriptyline-treated group ($p > 0.05$). Mean values of amitriptyline-, alendronate-, risedronate-, ibandronate-and etidronate-treated groups failed to show significant difference ($p > 0.05$) when compared to control group suggesting homogeneity among the groups.

Conclusion: Bisphosphonates appeared to have an antidepressant activity. More extensive research is required to substantiate and elucidate the role of bisphosphonates in behavioural disorders such as depression.

Keywords: Bisphosphonates, Depression, Amitriptyline

INTRODUCTION

Depression, a mental disorder, is often associated with receding bone mineral density (BMD). It has been reported that antidepressants, particularly in the aged population, are a cause of low bone strength and fractures [1, 2]. In addition, the antidepressants that target the serotonin system have been found to be detrimental for bone health and cause osteoporosis [3].

A relationship between depression and osteoporosis has become more evident over the years, as decreased BMD has been observed in depressed women and men. Compared to older women, aged men appear to be more susceptible to the effects of depression on BMD [4]. It has also been observed that depressed men have lower bone mass than non-depressed men [5]. Studies on animals have also suggested that depression may predispose to osteoporosis. Recent long-term studies have also shown an association between menopausal status with higher risk of depression and osteoporosis [6].

The positive effects of bisphosphonates, including improvement in anxiety or depression [7], are an area of research in the recent years. A study conducted by Kastelan *et al.* revealed that ibandronate treatment lead to significant improvement in mobility and the condition of depression [8]. Alendronate, on the other hand, significantly decreased the duration of immobility and increased the motor activity and thus can be assumed to play a role in reducing the symptoms of depression [9].

The application of bisphosphonates, especially risedronate, ibandronate and etidronate in the treatment of depression along with osteoporosis is limited. In addition, only few studies are available with respect to evaluation of alendronate in the treatment

of depression [10]. Therefore, the present study was undertaken to evaluate the effects of bisphosphonates, such as alendronate, risedronate, ibandronate and etidronate on depression in adult male Wister rats and Swiss albino mice

MATERIALS AND METHODS

Test animals

Adult male Wistar rats weighing 175±25 g and adult male Swiss albino mice weighing 25±5 g, housed under standard laboratory conditions, were obtained from the central animal house of J. N. Medical College, Belagavi. They were acclimatized to a photoperiod of 12 h for 10 d prior to the day of experimentation. Animals were provided feed (standard chow pellet) and water ad libitum. On experimental days, they were sustained on water, as the test drugs had poor intestinal absorption, especially in the presence of food [11]. Wistar rats and Swiss albino mice (36 of each type) were used for experimental purpose. The study was approved by Indian Animal Ethics Committee (IAEC) as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Chemicals

Antidepressant amitriptyline was procured from Merind Ltd. (Wockhardt Ltd.). The test antidepressants—alendronate and risedronate were obtained from Cipla Ltd., ibandronate and etidronate were procured from Sun Pharmaceuticals Ltd., and Vardhaman Health Specialities, Bangalore, respectively.

Dosage

The Wister rats and Swiss albino mice (36 each) were divided into six groups where one group was designated as control group and the

rest of the as test groups. Drug administration was not done in the control group. Each group comprised of six rats or mice. Antidepressants were administered orally by dissolving in distilled water. Clinical doses for these drugs were converted into rat or mouse-equivalent doses with the help of the table devised by Paget and Barnes (table 1) [12] and effect of each drug on animals was individually evaluated. A 24-h fasting period was observed prior to the start of the experiment.

Table 1: Number of groups, drugs and dosages used in the study

Groups	Treatment	Human clinical dose (mg)	Rat equivalent dose (p. o, mg/kg)	Mouse equivalent dose (p. o, mg/kg)
1	Control	–	0.5 ml	0.5 ml
2	Amitriptyline (Standard)	300	27	39
3	Alendronate	5	0.45	0.65
4	Risedronate	5	0.45	0.65
5	Ibandronate	2.5	0.225	0.325
6	Etidronate	5	0.45	0.65

p. o.: per orally

Behavioural studies

Antidepressant activity was assessed using the forced swimming test (FST) in Wistar rats and tail suspension test (TST) in Swiss albino mice. Amitriptyline was treated as the standard antidepressant drug for comparison. Locomotor activity was performed and simultaneous measurements of locomotion, exploratory behaviour and anxiety were taken. Behavioural studies were carried out in the afternoon (2:00 pm to 5:00 pm) under low illumination.

FST

The rats were more selective for this experiment, as they give less false positives and hence were preferred over the mice for this experiment [13]. The rats were subjected to a two-session test. The first session (pre-test) was conducted for 15 min prior to the drug administration with no behavioural recording. After 24 h, the test session was performed for 6 min in which rats were initially administered with the respective drug three times: at 24 h, 5 h and 1 h. The rats were then held in water maintained at 25±2℃ in a vertical Plexiglas cylinder (40 cm deep and 18 cm diameter). The duration of immobility (in seconds) was video recorded for each of the test drugs [13, 14].

TST

A modified version of the test was performed with an increase in the distance between the base and the snout of the mouse to enhance sensitivity of the test. Stress was induced in the test mice by suspending the animal by its tail with a distance of 58 cm between the base and the snout of the mice. Immobility time (in seconds) was recorded for 6 min [13].

Locomotor activity

It helps to rule out any influence of the drugs on locomotor system, which in turn may affect immobility. Pretreated mice were placed in the centre of the apparatus for 5 min. The device electronically counts the number of times the infrared beams are interrupted by movement of the animal, which in turn is the measure of the locomotor activity [15, 16].

Statistical analysis

Data, expressed as mean±SD, were analysed by using one-way analysis of variance (ANOVA) and Bonferroni's multiple comparison test [17] with the help of GraphPad Prism 4.0 software. $p \leq 0.05$ was considered as statistically significant.

RESULTS

Behavioural studies

FST in adult male wistar rats

Duration of immobility was significantly reduced in the standard and test drugs when compared to control group (fig. 1). Also, all the four test groups were found to be insignificant as compared to that of amitriptyline-administered group ($p>0.05$; table 2).
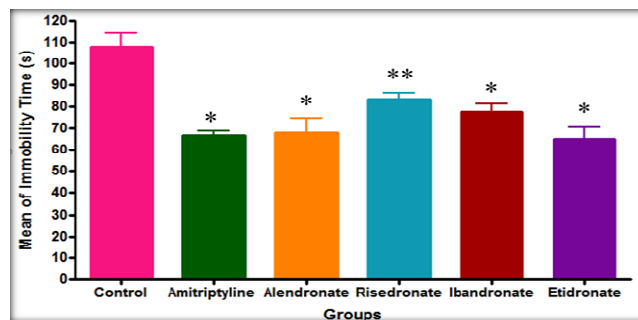

Fig. 1: Effect of various treatments on forced swimming test (*p<0.001 as compared to control; **p<0.05 as compared to control); data are represented as mean±SD

Table 2: Effect of various drugs in behavioural paradigms

Tests	Mean difference in seconds (s)					
	Control (n = 6)	Amitriptyline (n = 6)	Alendronate (n = 6)	Risedronate (n = 6)	Ibandronate (n = 6)	Etidronate (n = 6)
FST	108.2±14.8	70.17±7.13	63.17±24.47	72.83±31.15	77.83±8.23	52.67±27.00
TST	129.2±20.20	71.00±8.46	75.40±17.26	77.00±6.32	87.40±4.21	75.00±27.99
Locomotor activity	438.2±166.5	412.8±73.02	395.8±49.71	404.6±124.0	417.0±55.54	396.0±107.4

Data expressed as mean±SD; FST: Forced swimming test; TST: Tail suspension test

TST in male swiss albino mice

The standard, amitriptyline and the test groups showed significant decrease ($p<0.01$) in the duration of immobility when compared to control group (fig. 2). All the four test groups were found to be insignificant as compared to that of amitriptyline-treated group ($p>0.05$, table 2).

Locomotor activity

The mean values of amitriptyline-, alendronate-, risedronate-, ibandronate-and etidronate-treated groups failed to show significant difference ($p>0.05$) when compared to control group (table 2) suggesting homogeneity among the groups (fig. 3).
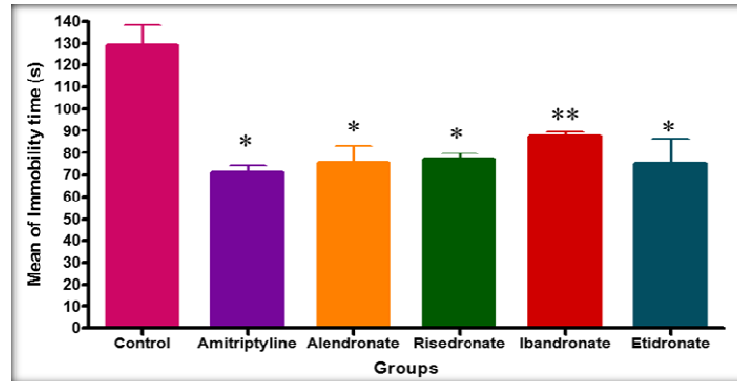

Fig. 2: Effect of various treatments in tail suspension test (**p*<0.001 as compared to control; ***p*<0.01 as compared to control); data are represented as mean±SD
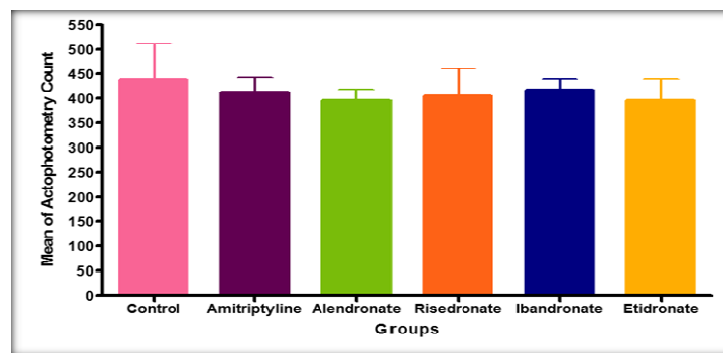

Fig. 3: Effect of various treatments on locomoter activity; data represented as mean±SD

DISCUSSION

Depression, one of the leading mental disorders, is widely associated with other diseases such as osteoporosis. As a result of depression and dysthymia, a decrease in BMD has been observed in men, as well as in adolescent girls and women [18, 19].

In the present study, antidepressant activities of alendronate, risedronate, ibandronate and etidronate were evaluated using experimental models of depression. The effects of these drugs on locomotor performance were also evaluated. FST in adult male Wistar rats and TST in adult male Swiss albino mice were performed to evaluate the antidepressant activity of the selected bisphosphonates.

The depression paradigms, FST and TST, were preferred because they were convenient and gave rapid results [16]. The results revealed that the administration of the selected bisphosphonates displayed lesser duration of immobility than the control signifying that these drugs can have antidepressant-like activity.

The effects of drugs on locomotor activity, verified using actophotometer test, displayed no significant difference among the six groups suggesting minimal influence of the drugs on the locomotor system. The actophotometer test is often selected, as it provides measures of locomotion [20]. It also helps to differentiate between the effects of sedative and stimulant drugs.

Bisphosphonates inhibit osteoclasts and have anti-apoptotic effect on osteoblasts. Consequently, they prevent bone weakening. Several studies have reported the decrease in cytokine level as a result of bisphosphonate activity [21–23]. Bisphosphonates, such as risedronate, have been reported to reduce the cytokine level to a significant level and are also involved in inhibiting bone resorption [23]. On the other hand, etidronate has been found to have inhibitory effect on the macrophages for the secretion of cytokine [24]. The anti-inflammatory activity of ibandronate has also been studied and verified by Bianchi *et al.* [25]. Elevated levels of cytokines are detected in depression and may lead to low BMD. It has been established that anti-inflammatory drugs help in reduction of depression symptoms [26]. Thus, reduction in the level of cytokines by bisphosphonates could be a possible mechanism to reduce depression and increase bone density.

Studies involving osteoporotic patients with rheumatoid arthritis (RA) were conducted within study periods of 1.5 y and 3 y to determine the inhibiting property of the intermittent cyclical etidronate on bone resorption or inflammatory changes. It was found that etidronate inhibited the synthesis of mediators related to inflammation, pain, and angiogenesis [27]. Thus, by reducing the mediators of inflammation, these drugs can be hypothesised to have antidepressant activity.

Depression is one of the common symptoms associated with hyperparathyroidism and hypercalcaemia, which in turn cause bone loss [28, 29]. As bisphosphonates are used to prevent the bone loss

in the body, they have also been found to reduce hypercalcaemia and hyperparathyroidism, although at different doses [30]. Although, few reports suggest that bisphosphonates, such as alendronate or risedronate, have adverse effects on primary hyperparathyroidism patients such as increase in the level of parathyroid hormone [31].

Although bisphosphonates are among the first choice of drugs for the treatment of bone disorders such as osteoporosis, they have also been found to cause several adverse effects [32]. Most important long-term side effects of this class of drug are osteonecrosis of the jaw, atrial fibrillation, renal failure, nephrotic syndrome, femoral fractures and severe suppression of bone turnover. Other most commonly reported short-term side effects include severe musculoskeletal pain, hypocalcaemia, oesophageal cancer, electrolyte imbalance and ocular inflammation [33, 34]. Even though several studies have stated these effects, there are the reports arguing against any definite link between the side effects and bisphosphonates. Some of the conditions may be due to long-term accumulation of the drugs in the body or some predisposing condition, such as chronic kidney disease present in the patient administered with bisphosphonates [35]. In cases of drug accumulation, patients are often put on 'drug holiday' in which the bisphosphonate therapy is stopped for a period of time [36]. Trials conducted also indicate that the risk of fractures is low even in women when bisphosphonates were administered for more than 10 y [37].

CONCLUSION

Bisphosphonates, such as alendronate, risedronate, ibandronate and etidronate have shown significant decrease in the duration of immobility as compared to the standard antidepressant drug—amitriptyline. However, further studies including the evaluation of their effect on biochemical profiles and stress levels are required to establish their role as antidepressant drugs.

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CONFLICT OF INTERESTS

The Author(s) declare(s) that they have no conflicts of interest to disclose

AUTHOR CONTRIBUTION

SST designed the study and prepared the manuscript. AN acquired and analysed the data and prepared the initial draft of the manuscript.

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