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EFFECT OF DIFFERENT DOSES OF OLANZAPINE ON MICE'S BONE TISSUE

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Summary

It has been a growing interest in side effects produced by the use of drugs that act on brain neurotransmitters such as serotonin, dopamine and norepinephrine. This study investigated the effect of olanzapine on mice's bone tissue. Fifteen (15) male adult mice were divided into three groups: Control (n = 5) without experimental dose, Group 1 (n = 5) with a dose of 15mg/Kg olanzapine and Group 2 (n = 5) with 20mg/kg of olanzapine. Group 2 had reduced body weight from the seventh day of treatment. Both Group 1 ($p = 0.013$) and group 2 ($p < 0.0001$) showed a reduction in the tibia weight. The length and thickness of proximal and distal epiphyses showed no statistical differences. The thickness of diaphysis was lower in group 2 ($p = 0.0025$) when comparing to the control, and when comparing the experimental groups, group 2 also showed a reduction in thickness ($p = 0.0259$). The microscopic analysis showed no change in cortical thickness of diaphysis; however, the number of osteocytes in the epiphysis of group 2 was lower ($p = 0.0461$). It was noted that the dose of 20 mg/kg showed significant differences in body and bones development of animals analyzed, in particular due to the lethargic state caused by the drug, reducing the ingestion of food and water and preventing their development.

Key words: Olanzapine, Atypical antipsychotics, Bone tissue, Tibia.

Introduction

Schizophrenia treatment has been enhanced by the introduction of atypical antipsychotics, such as clozapine, olanzapine and risperidone. These drugs have shown efficacy in reducing positive symptoms of schizophrenia and provide some improvement in negative symptoms and cognates. (1). In addition, atypical antipsychotics are reported since they reduce the incidence of extrapyramidal symptoms and tardive dyskinesia (2).

Despite all the additional properties favorable to atypical antipsychotics, there are also unfavorable clinical properties, which may include weight gain, sedation, seizures or agranulocytosis (3).

The use of classical antipsychotics has been suggested as a factor associated with osteopenia/osteoporosis in patients with schizophrenia, due to increased blood levels of prolactin caused by medicines (4-9). Treatment with these medicines causes the blockade of dopamine central receptor 2 (D2) and consequent hyperprolactinemia (6).

Studies show that atypical antipsychotics management has shown a reduction in the rate of osteoporosis in patients with schizophrenia. Atypical neuroleptics (such as olanzapine) block dopamine and serotonin receptors. As a result, the serotoninergic receptor antagonism promotes the reduction of hyperprolactinemia, the main factor associated with decreased levels of bone mineral density in schizophrenic patients (10,11).

Few studies have reported the impact on schizophrenia treatment with atypical antipsychotics on bone tissue. Studies showing this positive influence to prevent bone mineral loss are essential to prevent the appearance of symptoms and sequels in patients with mental disorders. Based on the lack of literary studies that evaluate side effects of olanzapine in the bone tissue, and the need for evaluation of potential consequences of its dosages, this study aimed to evaluate histometric changes in mice's tibias treated with different doses of olanzapine.

Methods

Animals

Fifteen adult (90 days) male mice (Mus musculus) were used from the National Agricultural Laboratory of Pernambuco (LANAGRO-PE). The animals were kept in a place with temperature of 23 ± 1° C in light/dark cycle of 12/12 hours, free access to food (Labina ®) and filtered water.

Experimental Design

Animals were randomized into three groups according to the experimental dose used: Control (CG) (n = 5) without experimental dose, Group 1 (G1) (n = 5) with 15 mg/Kg of olanzapine and Group 2 (G2) (n = 5) with 20mg/kg of olanzapine.

The treatment was conducted for 30 days with intraperitoneal application of the drug.

The experimental protocol was approved by the Ethics Committee for Animal Use (CEUA) of Federal Rural University of Pernambuco (CEUA - UFRPE - 14/2008) according to basic principles for research by using animals.

Methodological Procedures

During treatment, on days 01, 07, 14, 21 and 30, mice were weighed and anesthetized via intramuscular with Xylazine Hydrochloride (Rompum ® - Bayer) and Ketamine (Ketalar ®) (12). After euthanasia, animals' lower limbs were dissected and their tibias collected. The length (figure 01.A) and thicknesses of proximal and distal epiphysis (figure 01.B) and diaphysis thickness of the samples was measured with a digital pachymeter (Jomarca ®). Then, the tibias were weighed into a digital scale (AND HR 200 ®), fixed in buffered formaldehyde (10 ml of formaldehyde 37% and 27 ml of phosphate buffer 0.1M and pH = 7.0) at volume 50 times the sample volume and stored in glass containers. Next, the bone was demineralized with formic acid solution 90% and sodium citrate during the necessary time for this procedure. After decalcification, the tibia

was transversely sectioned, under the proximal epiphysis and level of the middle third of its diaphysis. Then, segments were placed in a neutralizing solution (Sodium Hydroxide, 5%) for 20 minutes and stored in 70% ethanol. Subsequently, the material was sent for routine histological processing, and embedded in paraplastic. The sections of 5µm thickness were stained with hematoxylin-eosin and mounted between slide and coverslip with synthetic resin (Entellan - Merck).

see Fig. 1

Microscopic Analysis

After preparing the slides, they were analyzed by light microscopy and images capturing was performed with a camera (Canon Powershot A470) attached to the eyepiece of the device with a 40X objective. The histometric analysis was performed by the photomicrographs obtained with the ImageJ software (National Institutes of Health, Bethesda, MD). In order to count the number of osteocytes, the tibial diaphysis was divided into three regions: lateral, intermediate and medial. In each region three microscopic fields were assessed, totaling nine fields per animal. For this assessment we used a millimeter lattice of 441 points and the cells located at the intersection points of the millimeter lattice were recorded (Figure 02).

see Fig. 2

In order to measure the cortical thickness of the tibial diaphysis, an ImageJ software (National Institutes of Health, Bethesda, MD) was also used. From the photomicrographs taken with the 10X objective, we determined points to 0°, 90°, 270° and 360°, and the measure was performed at these points. For each animal, four measurements at different sites were performed and then an average of measured values was calculated to obtain the average value of cortical thickness in the diaphysis region of these bones (13 - modified) (Figure 03).

see Fig. 3

Statistical Analysis

For the statistical treatment of results, a BioEstat 5.0 software was used and the Student t test was applied. We used a safety margin of 95% reliability in accordance with the sample and proposed objectives.

Results

On the seventh day of treatment there was a significant difference in animals' body weight when comparing the group treated with dose of 20 mg/kg and control; group 2 has shown a reduction in weight. The same trend was seen in the fourteenth, twenty-first and thirtieth day of treatment (Table 01). However, group 1, despite showing a trend to reduce weight, did not show significant difference in relation to the control.

see Table 1.

The table 02 shows data on differences between groups regarding weight (in grams) of tibias and number of proximal epiphysis osteocytes. We also observed that there was no interference from different pharmacological dosages on cortical thickness of the diaphysis (in micrometers). 88

see Table 2.

As seen in the figure 04 (A, B and C), there was no interference of Olanzapine in length and thickness parameters of proximal and distal epiphysis in different groups. However, there was a significant difference in the tibial diaphysis thickness between animals of CG and G2 as well as when comparing animals of G1 and G2 (Figure 04 D).

see Fig. 4

Discussion

This study demonstrated that there's a reduction in animals' body weight subjected to higher doses

of Olanzapine in relation to the control. This fact may have occurred due to the lethargic state of those animals several days after the drug application. During observation periods of those animals' behavior in their cages, it was evident that they did not seek food or water with the same avidity observed in other groups. This sedation state, which is an expected effect due to the use of Olanzapine (3), and the fact that they didn't seek food seem to have been the main reason for the reduction in animals' body weight of group 2. A poor diet also appears to have affected the individual weight of mice's tibias.

Non nutrients intake, in particular, calcium, sodium, magnesium and potassium, in normal conditions, may lead to improper replacement of the bone matrix, by resulting in a decrease in weight of tibias evaluated. This fact contradicts other studies made by Czobor et al (14) and Simpson et al (15), who reported weight gain and body mass index gain (BMI) in patients subjected to treatment with Olanzapine. Allison et al. (16) demonstrated that in humans, Clozapine and Olanzapine are atypical antipsychotics that cause more weight gain in 10 weeks. This weight gain may be related to the fact that Olanzapine acts by blocking dopamine and serotonin receptors, inducing an increase in appetite (17) and decreased movement due to the sedative effect of the drug. This contradiction in relation to our study may be due to different doses used and, especially, the various individuals involved in the studies.

Parameters related to the length and thickness of proximal and distal epiphyses of the tibia do not seem to be influenced by Olanzapine. However, the diaphysis thickness was reduced in animals belonging to the group of higher Olanzapine dosage when compared to the group of 15 mg/kg and control. It is likely that this difference is related to a greater pressure that the bone portion is subjected to both downwardly due to the animal's weight, and upwards, due to a ground reaction.

Occasionally, the bone tissue has a capacity for development, depending on the mechanical stress to which it is being subjected to. As the Olanzapine acts on mice, making them lethargic, without their proper movement, the lack of mechanical stress on bones possibly decreased. This may have affected the bone diaphysis development, which depends on this mechanical stress. As the treatment did not last long, the Olanzapine interference reflected only on the tibial diaphysis thickness. It is possible that other bone parameters showed significant changes if animals were subjected to treatments of longer duration.

Microscopic analysis of slides showed that the Olanzapine did not affect the cortical thickness of the tibial diaphysis. However, there was a significant reduction in the number of osteocytes for animals of group 2 when compared to the control, suggesting a negative interference of Olanzapine in bone tissue's cells. Some clinical studies have also demonstrated changes in blood cells such as leukopenia, granulocytopenia, neutropenia, thrombocytopenia and anemia resulting from the use of Olanzapine (18-23), thus confirming our results.

The Olanzapine also interferes in metabolic processes, e.g. in glucose control, leading patients to diabetes mellitus. This process is still not fully understood (24,25). Glucose is essential for cell replication. As the glucose transport to cells in animals treated with Olanzapine is affected, it is likely that this replication suffers losses, resulting in a decreased number of osteocytes. This fact may also relate to the parameter previously analyzed (tibial diaphysis thickness). Although the number of osteocytes of tibial diaphysis was not measured in this study, it is likely that the reduction in the epiphysis has also occurred in the diaphysis osteocytes, leading to a deficiency in the bone matrix maintenance and osteocytes function, thus resulting in a reduction in the diaphysis thickness of animals treated with Olanzapine.

Parameters related to body weight, weight and thickness of the tibial diaphysis and number of osteocytes were modified in mice subjected to treatment with Olanzapine, especially in high dosages. This medicine management seems to

induce a sedation state, which may reduce the adequate ingestion of food by those animals, thus interfering in the maintenance and remodeling of bone tissue.

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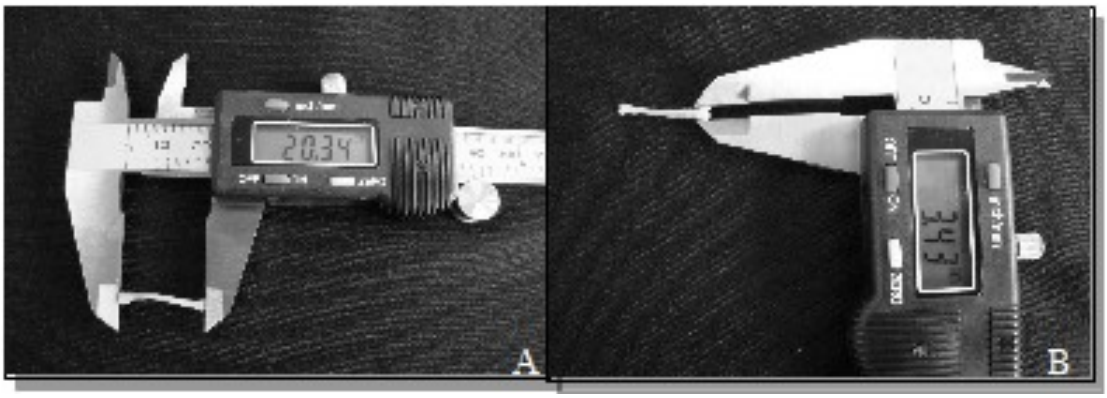

Figure 1 - Measuring the length (A) and thickness (B) of the proximal and distal epiphysis with a digital pachymeter.

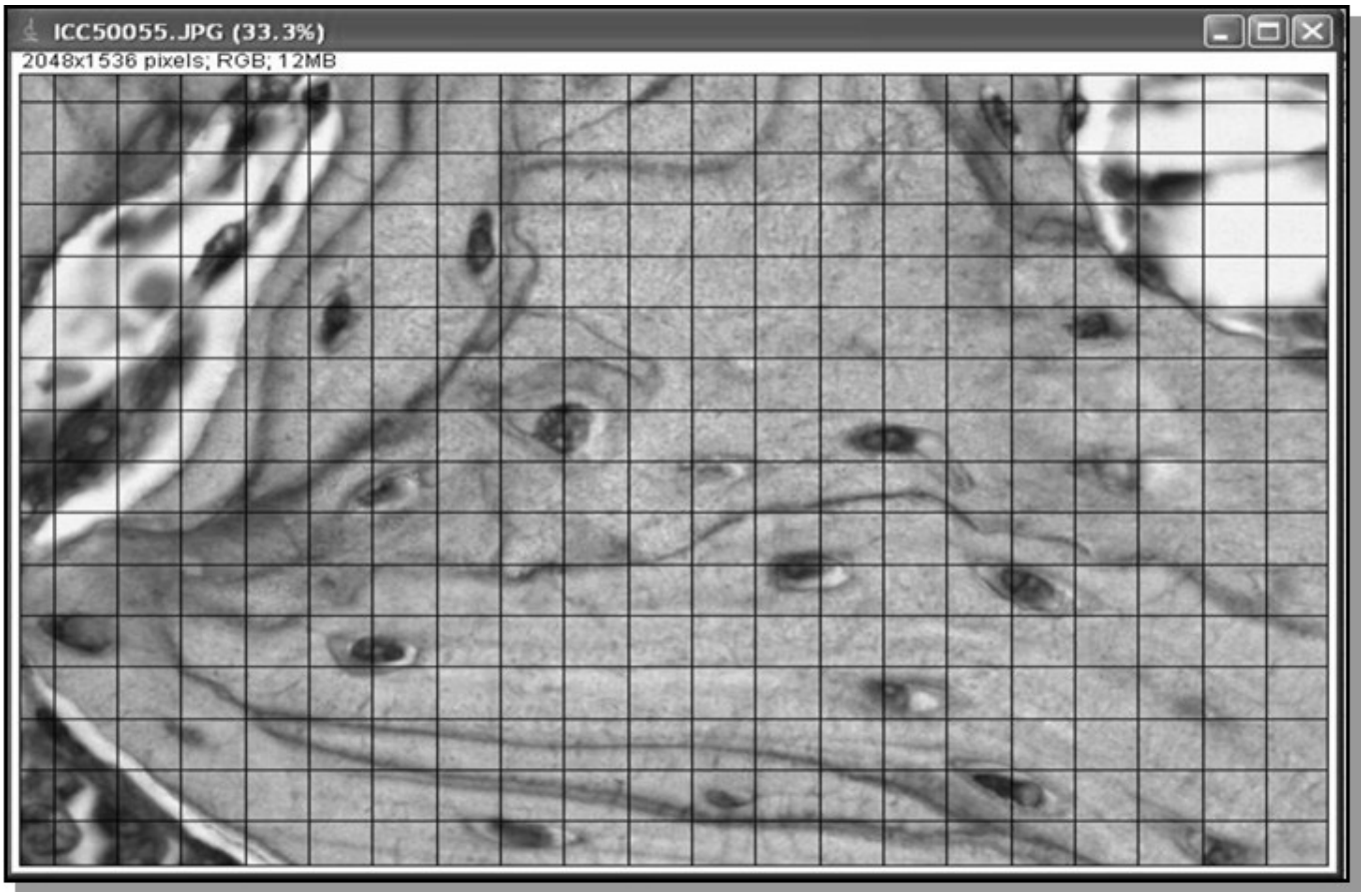

Figure 2 - Counting the number of osteocytes with a millimeter lattice of 441 points.

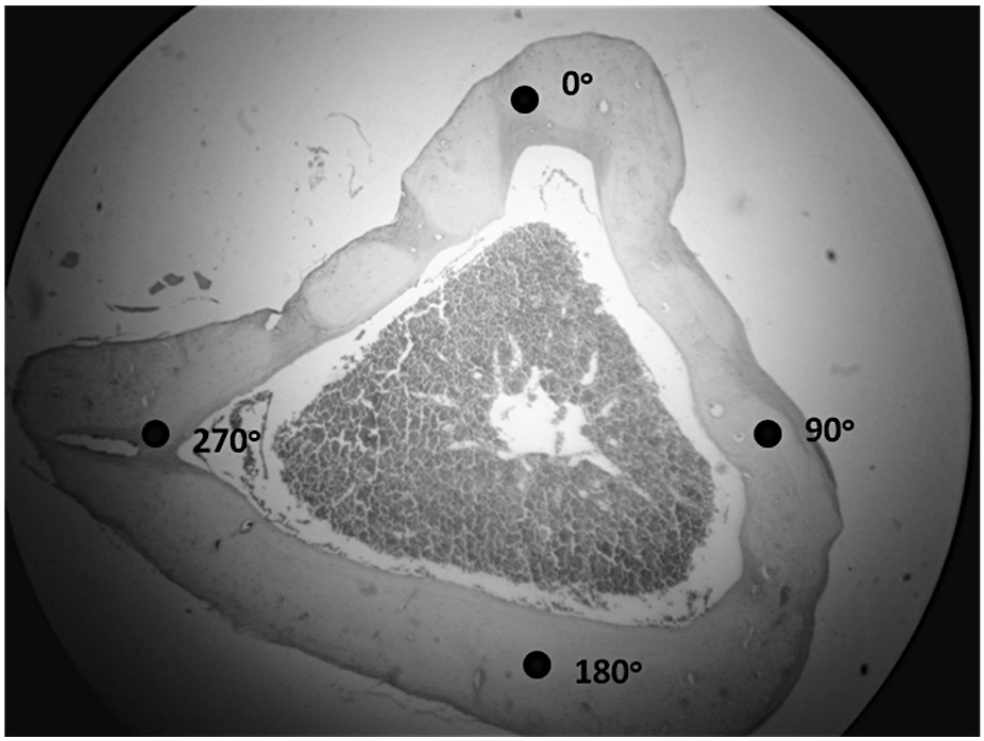

Figure 3 – Measuring the cortical thickness of tibial diaphysis.

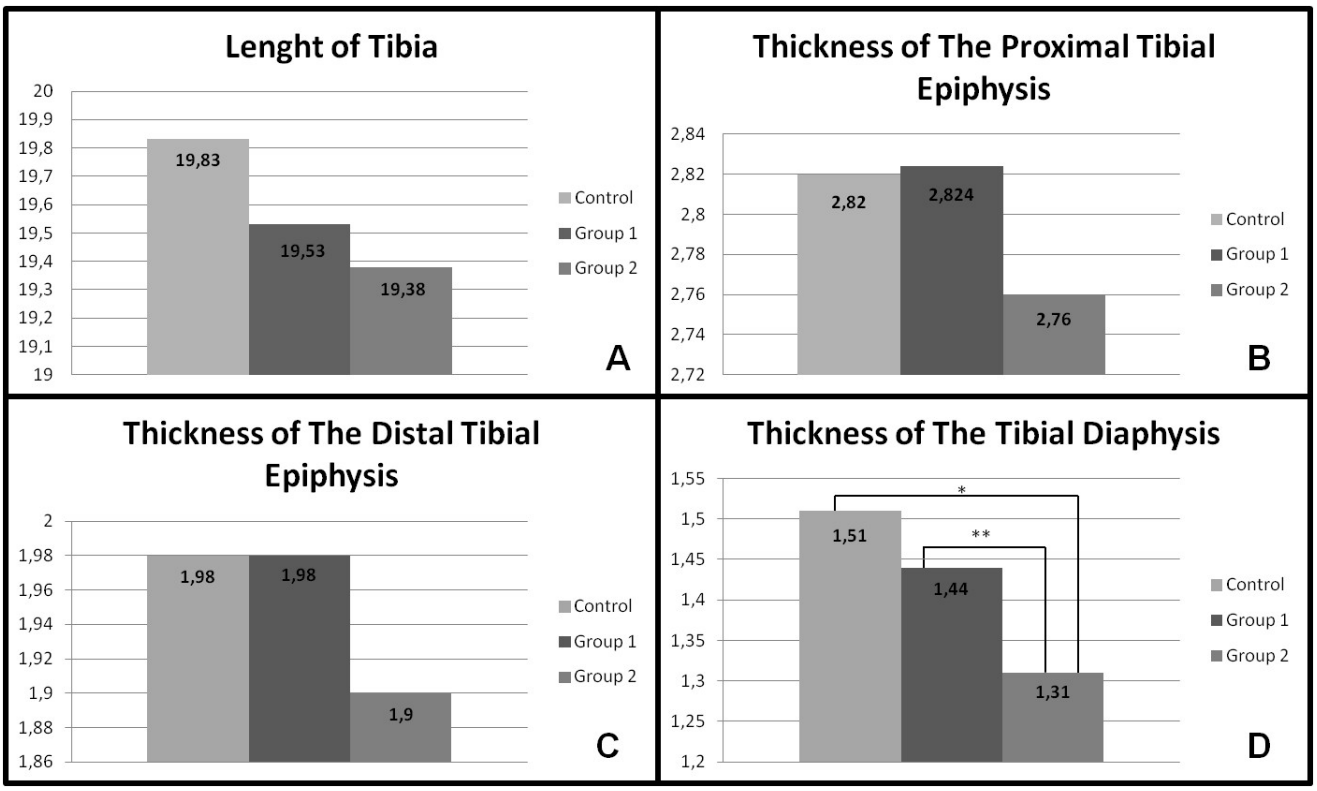

Figure 4 - Graphs showing the average values for length and thickness of the proximal and distal epiphysis and thickness of the tibial diaphysis.

* Statistical difference between the control and group 2
** Statistical difference between the group 1 and group 2

Days	Control (n = 6)	Group 1 (n =5)	Group 2 (n =5)	p
1	28,1 ± 4,1	33,4 ± 1,9	31,6 ± 1,9	0, 0589
7	34,8 ± 3,2	31,8 ± 2,3	29,6 ± 1,8	0, 0281[a]
14	37,1 ± 4,8	33,2 ± 3,5	30,0 ± 1,8	0, 0413[b]
21	38,5 ± 4,3	33,8 ± 2,8	30,6 ± 2,0	0, 0162[c]
30	41,0 ± 3,6	37,0 ± 3,3	31,8 ± 2,7	0, 0084[d]

Table 01 – Means and standard deviation of weights, in grams, of mice during 30 days of treatment with different doses of olanzapine.

[a] Statistical difference between the control and group 2
[b] Statistical difference between the control and group 2
[c] Statistical difference between the control and group 2
[d] Statistical difference between the control and group 2

Parameters	Control (n = 6)	Group 1 (n =5)	Group 2 (n =5)	p
Tibia Weight	0,088 ± 0,0055	0,078 ± 0,0061	0,066 ± 0,0025	0,013[a]
Cortical Thickness of Diaphysis	186,46 ± 61,37	160,86 ± 24,18	213,91 ± 58,83	<0,0001[b]
Number of Osteocytes	124,33 ± 24,54	107,20 ± 9,47	106,80 ± 10,16	0,0461[c]

Table 02 – Means and standard deviation of tibia weight (in grams), cortical thickness of diaphysis (μm) and number of osteocytes.

[a] Statistical difference between the control and group 1
[b] Statistical difference between the control and group 2
[c] Statistical difference between the control and group 2