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Impact of Melatonin in Comparison to Bisphosphonates on Induced Osteoporotic Alveolar Bone in Albino Rats

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ORIGINAL STUDY

Impact of Melatonin in Comparison to Bisphosphonates on Induced Osteoporotic Alveolar Bone in Albino Rats

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Abstract

Purpose: This study aimed to assess the effect of melatonin in comparison to bisphosphonates on induced osteoporotic alveolar bone of albino rats. Material and methods: In all, 28 female albino rats were used in this study. The rats were divided into four equivalent groups (8 rats each) as follows: group I: normal control group, group II: glucocorticoid-induced osteoporotic group (GIO) (dexamethasone 7 mg/kg/week intramuscularly), group III: alendronate group; the rats received glucocorticoids in addition to alendronate (2 mg/kg daily orally). Group IV: melatonin received (7 mg/kg/week IM) and melatonin (5 mg/kg/day subcutaneously). After 8 weeks, animals were killed and the blood was withdrawn for biochemical analysis, then the mandibular bone was taken for histological examination and for energy-dispersive X-ray (EDX). Results: In the GIO group: there was decrease in serum calcium, phosphorus, alkaline phosphatase (ALP), and osteocalcin (OC) concentrations and also a decrease in mineral content in the tissue. Also, by light microscope this group showed bone degeneration. Using melatonin and alendronate improved this degenerated change, but this improvement is better in melatonin than alendronate. Conclusions: Melatonin is more effective in the restoration of bone architecture than alendronate in glucocorticoid-treated rats.

Keywords: Alendronate, Alveolar, Melatonin, Osteoporosis

1. Introduction

Bone are not quiescent structures in the body; they undergo physiological changes by a process called bone remodeling. It is based on the equilibrium between bone formation and bone resorption [1].

Glucocorticoids are used for the treatment of inflammation, but they have several complications such as osteoporosis, as they elevate bone resorption through stimulation osteoclasts. They also decrease bone formation through the inhibition of osteoblasts and osteocytes [2].

Osteoporosis is a disease that occurs when the bone mineral density and the bone mass decrease due to changes in bone microstructure, finally resulting in low-impact, fragility fractures [3].

Consequently, using bisphosphonates (therapies used in the treatment of osteoporosis) enhance bone density and decrease fractures by 30e65 %. Alendronate is a type of nitrogenous bisphosphonate that acts by hindrance of bone resorption and increases bone strength. Treatment by alendronate may lead to several complications such as osteonecrosis of the jaw [4,5].

Melatonin is a hormone delivered by the pineal gland. The secretion of melatonin is controlled by the biological clock. Shift work, aging, or light at night makes disruptions in the biological clock which results in bone loss and elevation of fracture risk [6].

Moreover, deficiency in melatonin leads to an increase in bone resorption, which may be attributed to inhibitory effect on osteoclast function.

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Melatonin decreases osteoporosis in mice by lowering the number of osteoclasts and alkaline phosphatase (ALP) declaration. Melatonin also enhances bone metabolism and oxidation in osteoporosis [7].

This work aimed to assess the effect of melatonin in comparison to bisphosphonates on the induced osteoporotic alveolar bone of albino rats.

2. Materials and methods

2.1. Experimental animals

In all, 28 healthy female Albino rats weighting (250e300) grams were used in this study. They were obtained from the animal house of Zagazig University. All animals were capped and supplied with a regular diet and drinking tap water, and maintained under proper conditions of good ventilation during the whole experimental period. This study followed the rules and regulations of the animal's experimental studies certified by the Ethics Committee, Faculty of Dental Medicine for Girls, Al Azhar University including their facilities, diet, and method of euthanization (REC-BI-22-02).

2.2. Study design

Depending on a previous study [8], which found that the mean ± SD of BMD in methylprednisolone (reference group) and sodium alendronate were 0.211 ± 0.006 and 0.227 ± 0.008, respectively, and setting the power = 0.80 and a = 0.008 (to adjust for comparisons of four groups), the lowest sample size for an equal size a four group clinical trial is seven in each group as follows:

(1) Group I, (control normal group) (CN):

Rats of this group received a regular diet with free access to water and did not receive any medications.

(2) Group II (glucocorticoid-induced osteoporosis (GIO)):

Rats of this group received dexamethasone (Sigmatec Pharmaceutical Industries, Egypt) (7 mg/kg/week) by intramuscular injection for 8 weeks [9].

(3) Group III (alendronate) (ALN-GIO):

Rats of this group received dexamethasone (7 mg/kg/week) by intramuscular injection for 4 weeks followed by sodium alendronate ((Fosamax) Merck Sharp and Dohme, Italy) (2 mg/kg/day) orally by oral gavage for another 5 weeks with concomitant continuation of the weekly dexamethasone administration [10].

(4) Group IV (melatonin) (M-GIO):

Melatonin-supplemented osteoporotic group (M-GIO, n = 7): rats of this group received dexamethasone (7 mg/kg/w IM) for 4 weeks, followed by melatonin (Sigma Aldrich, Co.,3050 spruce Street, Louis, MO, USA) (5 mg/kg/day) by subcutaneous injection for 5 weeks with concomitant continuation of the weekly dexamethasone administration [11].

2.3. Euthanasia of experimental animals

In the end of the study, rats were anesthetized by ether and killed. Blood was collected from the aorta for biochemical analysis, then the mandible was divided into two halves. The right half was prepared for light microscopic examination, while the left half was prepared for energy-dispersive radiography (EDX) microanalysis.

2.4. Biochemical studies

After blood collection, each sample was centrifuged at 4 °C for 15 min at 3000 rpm, and the serum was collected and stored at —20 °C for serum calcium (Ca), phosphorus (ph), alkaline phosphatase (ALP), and osteocalcin (OC) estimation.

3. Results

3.1. Histological fIndings by light microscope

Group I (the control group)

The alveolar bone showed normal architecture, regular surface lined by a continuous layer of osteoblasts, and many deeply stained resting lines. The spongy bone showed dense bony trabeculae surrounding normal sized, cellular, and highly vascularized bone marrow spaces. Bone marrow spaces were lined by normal flat endosteal cells with deeply stained flattened nuclei. Bone trabeculae showed normal size and distribution of osteocyte lacunae. (Fig. 1(IA and IB))

Group II (glucocorticoid group)

The alveolar bone showed an irregular and scalloped surface. The bone showed marked thinning and discontinuity of surface osteoblasts. Also, large multinucleated osteoclasts were observed. Darkly stained reversal lines were also noted. Also the bone trabeculae showed decreased number of osteocyte,

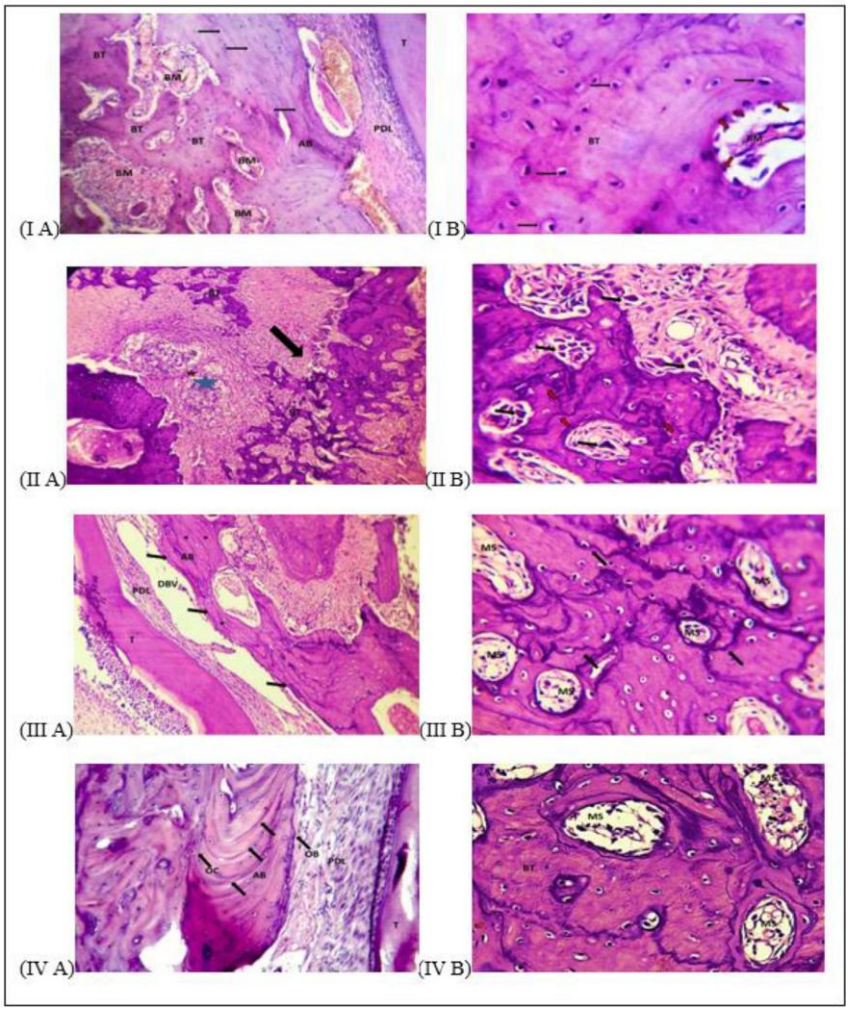

Fig. 1. A photomicrograph of the alveolar bone: (IA) control group showing: relatively regular bone surface facing the periodontal ligament with parallel resting lines (arrows), (IB) higher magnifIcation of (IA) showing the bone marrow space lined by normal flat endosteal cells with deeply stained flattened nuclei (red arrows) and bone trabeculae containing normal size and distribution of osteocyte lacunae. (black arrows) and dense bone trabecule surrounding normal size bone marrow spaces. (IIA) glucocorticoids group showing:destruction to PDL and thin bone trabeculae (arrow) and secondary infection,inflammatory reaction (star). ((IIB) the spongy bone showing Multinucleated osteoclas (black arrows) and darkly stained reversal lines (red arrows). (IIIA) alendronate group showing: relatively regular bone surface with interrupted arrangement of osteoblasts (arrows). (IIIB) the spongy bone showing dense bone with numerous relatively normal osteocytes and normal marrow spaces.with some reversal lines (arrows). (IVA) Melatonin group showing:regular bone surface facing PDL. Note resting lines (arrows)., numerous flattened osteoblasts lining the surface of bone (OB).also containing numerous relatively normal osteocytes (oc), (IVB) the spongy bone showing thick bone trabeculae surrounding normal bone marrow spaces. T (tooth), PDL (periodental ligament), BT (bone trabeculae), AB (alveolar bone), MS (marrow space), DBV (dilated blood vessel).

pyknotic nuclei, empty lacunae bone degeneration, and edema in some areas. In addition destruction of the periodontal ligament (PDL) and the bone were replaced by an acute inflammatory reaction, consisting mainly of polymorphonuclear leukocytes (neutrophils) and monocytes (acute osteomyelitis). Cancellous bony trabeculae were notably irregular, thin, and scalloped, surrounding wide bone marrow spaces. (Fig. 1(IIA and IIB))

Group III (alendronate-treated group)

The alveolar bone in this group revealed many of the normal features of the alveolar bone, which were relatively restored. The surface of the bone beside the PDL was found relatively regular with discontinuity in the arrangement of surface osteoblasts; parallel darkly stained resting lines were observed. Numerous enlarged osteocytes lacunae

were evident. Areas of newly formed bone also were observed. Areas of spongy bone showed dense bone trabeculae surrounding relatively normal sized bone marrow spaces. (Fig. 1(IIIA and IIIB))

Group IV (melatonin-treated group)

The bone architecture was relatively restored. The bone surfaces beside the PDL exhibited a smooth regular outline lined by a continuous arrangement of osteoblasts. In addition, normal size and distribution of osteocytes were noticed. Parallel intensely stained resting lines were noted. Cancellous bony trabeculae were dense comprising cellular, normal sized bone marrow spaces lined by endosteal cells with reversal lines occasionally observed. Normal size and distribution of osteocytes lacunae were also observed. (Fig. 1(IVA and IVB))

3.2. Results of EDX microanalysis

Table 1 shows comparison among the four groups regarding percentages of calcium and phosphorus. Calcium level in group II (GCs) was significantly lower than the control group I. However, levels of calcium in groups III (ALN) and IV (MLT) were significantly higher than group II. Phosphorus levels were inversely proportional to calcium levels; moreover, the differences between the four groups were statistically significant (Table 1).

3.3. Biochemical analysis

Mean and standard deviation (SD) values of biochemical analysis for all groups are shown in Table 2. It was shown there was a significant difference between groups in serum calcium. The highest value was found in group I, followed by group III, then group IV, while the lowest value was found in group II. In addition, there was significant increase of serum P in group III and group IV, while the value decreased in group II. While in serum ALP, there was no significant difference between groups (P = 0.084). In osteocalcin (oc), there was a significant difference between groups. The highest value was found in group (I), followed by group (IV), then group (III), while the lowest value was found in group (II) (Table 2).

4. Discussion

The most frequent secondary cause of osteoporosis is glucocorticoid-induced osteoporosis (GIOP), and the fractures that follow from it are very morbid. Within a few months of starting oral glucocorticoids (GCs), there was fast bone loss and an increase in fracture risk that is dose dependent [12].

Bisphosphonates are considered the best choice drug in the treatment of GIOP; Risedronate and alendronate had become the standard care of GIOP. However, prolonged use of these drugs may result in jaw osteonecrosis specially when combined with the intake of GCs [13].

The current study therefore evaluated the impact of melatonin in comparison to bisphosphonates on the induced osteoporotic alveolar bone of albino rats. The laboratory rat was used as a model in this study because it is convenient, easy and safe to handle, and cost-effective and is the most frequently used animal model for glucocorticoid-induced osteoporosis followed by mice. Dexamethasone was used to induce osteoporosis as it was reported that it has the most potential effect in the induction of osteoporosis [14]. The dosage and interval used to induce systemic osteoporosis was documented to induce generalized osteoporosis [9].

Histological findings of the control group showed normal architecture, a regular surface lined by a continuous layer of osteoblasts, and many deeply stained resting lines. The spongy bone showed dense bony trabeculae surrounding normal-sized, cellular, and highly vascularized bone marrow spaces. Bone marrow spaces were lined by normal flat endosteal cells with deeply stained flattened nuclei. Bone trabeculae contain normal size and distribution of osteocyte lacunae. These findings agreed with previous data describing normal structure of the alveolar bone [14].

Moreover, observation of the glucocorticoid group showed obvious bone surface irregularities and numerous large multinucleated osteoclasts. Cancellous bony trabeculae were irregular, thin, and scalloped; surrounding wide bone marrow spaces. Also, the bone trabeculae showed decreased number of osteocytes, pyknotic nuclei, empty lacunae bone degeneration, and edema in some areas. These results agreed with the previous study,

Table 1. Energy-dispersive radiography analysis results.

	(mean ± SD)					
Element	Group I	Group II	Group III	Group IV	f-value	P-value
Ca	65.37 ± 2.59^{B}	57.26 ± 3.75^{C}	69.57 ± 2.66^{A}	64.55 ± 7.46^{B}	24.07	<0.001*
P	34.63 ± 2.59^{B}	42.74 ± 3.75^{A}	30.43 ± 2.66^{C}	35.31 ± 7.67^{BC}	23.48	<0.001*

Table 2. Biochemical analysis.

	(mean ± SD)					
Measurement	Group I	Group II	Group III	Group IV	f-value	P-value
Ca++ (mg/Dl)	10.00 ± 0.44^{A}	7.47 ± 0.94^{B}	9.67 ± 0.45^{A}	8.57 ± 0.45^{AB}	10.68	0.004*
PO4 (mg/dL)	3.82 ± 0.87^{AB}	2.30 ± 0.40^{B}	4.17 ± 0.32^{A}	3.20 ± 0.80^{AB}	4.87	0.033*
ALP (U/L)	162.33 ± 43.25^{A}	75.00 ± 24.58^{A}	126.33 ± 52.88^{A}	157.33 ± 28.10^{A}	3.19	0.084ns
Osteocalcin (ng/mL)	14.23 ± 2.77^{A}	5.76 ± 1.60^{B}	12.97 ± 2.35^{A}	13.33 ± 0.61^{A}	11.40	0.003*

anddemonstrated that in the glucocorticoid-induced osteoporosis group, tibias showed thin bone trabeculae and increased intratrabecular distance. Decreased number of osteocytes inside lacunae and increased number of empty lacunae were also noticed in bone tissues, indicating osteocyte death [15].

Glucocorticoids have been known to reduce bone formation and enhance bone resorption by decreasing alkaline phosphatase and osteocalcin, bone formation markers. Furthermore, GCs extend the life span of osteoclasts and, in contrast, decrease the life span of osteoblasts. In addition, they impair osteoblast differentiation and osteoblastogenesis [16].

Alendronate is potent in the treatment and prevention of glucocorticoid-induced osteoporosis. In this study, levels of bone formation markers suggested stimulation of bone formation in the alendronate-treated group as there was an increased level of serum ALP, OC, Ca, and P as compared with the GIO group. Alendronate works by inhibiting the osteoclast function [17].

Many studies have demonstrated that bisphosphonates have the ability to inhibit osteoclast-mediated bone resorption. Alendronate treatment improved GIO in rats as it increased bone strength, bone mineral content, and improved histological damage of the bone matrix in the head of the femur [18].

The current histological results also revealed that melatonin was comparable to the bisphosphonate group preserving the normal bone architecture. The alveolar bone surfaces beside the periodontal ligament exhibited a smooth regular outline lined by continuous arrangement of osteoblasts. In addition, normal size and distribution of osteocytes was noticed. Parallel intensely stained resting lines were also noted. Cancellous bony trabeculae with dense comprising cellular, normal sized bone marrow spaces lined by endosteal cells with reversal lines were occasionally observed. Normal size and distribution of osteocyte lacunae were also observed.

The present findings were certified by a previous study, which reported that melatonin is efficient in regulating bone density by stimulating osteoblasts, preventing osteoclast differentiation and decreasing the process of bone resorption [19].

Melatonin causes elevation in urinary deoxypyridinoline, serum phosphorus, and bone alkaline phosphatase (BAP) in ovariectomized rats compared with untreated rats [20].

The EDX microanalysis showed a decrease in serum calcium content in the glucocorticoids group in comparison to the control group. These results are similar to other studies that reported a decrease in both the cortical and trabecular mineral content of bone on glucocorticoid treatment. Also, there have reported changes in body minerals of rats after dexamethasone administration [21,22].

Moreover, the EDX microanalysis showed that the Ca level significantly increased in the bisphosphonate group in comparison to other groups. These results were supported by other studies that reported that after treatment with alendronate, the bone mineral content and bone density in patients with osteoporosis were increased [23].

In addition, EDX microanalysis for the melatonin group showed an increase in calcium in comparison to the GC group, and this indicates that the melatonin enhances minerals of the bone. Those results agree with a previous study, which found that melatonin can enhance bone mass by stimulating matrix mineralization [24].

There is improvement in bone architecture in the melatonin group compared with the alendronate group, despite high mineral content of tissue in alendronate, may be attributed to the action of bisphosphonate on bone turnover as the bisphosphonate act by decreasing bone resorption, but at the same time decreases bone turnover, while melatonin act on both inhibition of bone resorption and stimulation of new bone deposition [25,26].

Glucocorticoid-induced osteoporosis group demonstrated a significantly decreased serum Ca and P as compared with the control group. This can be explained as GCs decrease calcium resorption at the renal tubule and calcium absorption in the bowel through a vitamin D-independent mechanism [27].

Moreover, GCs also decrease ALP and OC in comparison to the control group, suggesting

reduced bone turnover. Similar findings were revealed by a previous study, where the intake of GCs decreased serum ALP and OC levels [28].

4.1. Conclusions

The intake of glucocorticoids causes remarkable loss of the alveolar bone. Treatment with either melatonin or alendronate restores the micro-architecture of bone of GIO rats to different degrees, but melatonin is more effective in the restoration of bone architecture than alendronate.

4.2. Recommendations

(1) Further studies are recommended by the mechanism of action of melatonin at the molecular level, the pathways, as well as the signals involved.
(2) Further studies could be performed to evaluate the effect of bisphosphonates and melatonin on the PDL and tooth structure of GIO rats.

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Conflicts of interest

The authors have no conflicts of interests to declare.

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