

BIOLOGICAL EFFECT OF GLUCOCORTICOID ADMINISTRATION ON THE STRUCTURE OF ALVEOLAR BONE

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ABSTRACT

BACKGROUND: Glucocorticoids is a potent anti-inflammatory which is used to treat autoimmune disorders. Also, its used as an immunosuppressive agent. It also has an important role in calcium homeostasis. Hypocalcemia is a common metabolic disorder that may develop due to certain pharmacological agents used in the treatment of other diseases. Drug-induced hypocalcemia may vary in severity from asymptomatic to morbidity.

MATERIALS AND METHODS: Twenty albino adult male rats whose weight ranges from 200-250 grams (about 6 months of age) were included in this study. They were divided randomly into 2 groups: Group I (control group), Group II (glucocorticoid group). Rats in group (II) were injected by methylprednisolone (40 mg/kg) intramuscular 3 times per week for three weeks. The animals were euthanized by the end of the experimental periods, after 3 weeks. The right molar segments were dissected out and prepared for light microscopic examination while the left molar sections were processed for energy dispersive x-ray microanalysis (EDX) and scanning electron microscope (SEM).

RESULTS: The results of control group showed normal histological features of the alveolar bone. Regarding the glucocorticoid group, there was disturbance in the bone architecture. The bone surface showed an irregular outline with multiple resorptive craters and porosity. Deeply stained incremental lines were also evident. The results of EDX showed decrease in calcium and phosphorous percentage in group B than in group A.

CONCLUSION: Glucocorticoid administration is a serious condition which leads to severe bone loss and alteration in structure of alveolar bone.

KEYWORDS: Calcium, Glucocorticoids, Calcium deficiency.

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INTRODUCTION

Glucocorticoids are steroid hormones that are extensively used in inflammatory diseases and as immunosuppressive drugs to treat autoimmune disorders as well as allergic reaction, skin diseases, asthma and hematological disorders (1, 2).

Furthermore, glucocorticoid perform its action through two different mechanisms: the genomic and the non-genomic pathway. The genomic pathway is provoked by glucocorticoids (GC) binding to glucocorticoid receptor (GR). Once it reaches the cytosol, GCs bind strongly to the cytosolic GR in order to become activated. Afterwards, GC-GR

complex is translocated to the nucleus, where it binds to a certain DNA sequence, glucocorticoid response elements (GREs) (3, 4).

Glucocorticoids- glucocorticoid receptor complex and GRE interact and may activate (transactivation) or repress (transrepression) transcription. Transrepression prohibit many molecules that are pro-inflammatory and it encounters for the anti-inflammatory and immunosuppressive actions of GCs. However, transactivation mediates transcription of many enzymes that are linked to gluconeogenesis and the adverse effects related to GCs (5, 6).

However, in the non-genomic pathway GCs and cytosolic glucocorticoid receptor interact in a glucocorticoid receptor-dependent but transcriptional-independent mechanism (7). This is illustrated due to the binding of GC-GR complex which lead to interaction with DNA through the classic genomic mechanism and with intracellular proteins, leading to inhibition of inflammatory mediators as arachidonic acid. Additionally, Glucocorticoids mediate their actions through interaction with biological membranes, especially, mitochondrial and cellular membranes. By this mechanism, GCs is able to decrease ATP that the immune cells need to maintain their functions (4).

Glucocorticoids are suggested to play a crucial role in maintaining calcium homeostasis. It is suggested that glucocorticoid-induced calcium malabsorption is due to suppressed expression of calcium-processing genes (8). It has been shown that glucocorticoids affect intracellular calcium concentration physiologically and pathologically. The application of exogenous glucocorticoids lead to reduction in calcium ions in the hypothalamic neurons (9).

Calcium as an element abundant in the body which is commonly involved in bone building and metabolism. About 99% of total calcium in the body is present as hydroxyapatite crystals (HA) in bones and teeth, where it strengthens hard tissue and about 1% of this calcium is freely commutable with the extracellular fluid. Calcium in the extracellular fluid, circulatory system, muscles, and other tissues, is important in vasoconstriction and vasodilation and in mediating muscle function, neurotransmission, intracellular signaling, and endocrine secretions (10, 11).

Calcitonin, parathyroid hormone and vitamin D altogether sustain the concentration of serum calcium at a nearly constant level. They control calcium absorption in the gut, renal reabsorption, calcium excretion, and the use of calcium stores in the bone (11).

Hypocalcemia or Calcium deficiency is a common electrolyte imbalance that occur in many patients and it ranges from mild to critically severe illness. Hypocalcemia is known as a decrease in the total serum calcium level below 8.5 mg/dl (2.12 mmol/l) (12).

Hypocalcemia occurs when the net outflow of calcium from the extracellular fluid is more than that can be replaced from the bone or intestine due to disturbances in any of the calcium regulatory mechanisms (13).

Moreover, many drugs as bisphosphonates, antiepileptics, proton pump

inhibitors and diuretics, have been involved in the etiology of hypocalcemia. Nevertheless, since calcium deficiency is a multifactorial disease, the diagnosis of drug-induced calcium deficiency maybe missed and can lead to morbidity and death (14).

Bone is a rigid, cellular structure that is made of 67% inorganic (hydroxyapatite crystals) and 33% organic content (collagenous and non-collagenous proteins). The mineral composition is hydroxyapatite crystals with traces of other elements. Bone structure is maintained by a process called bone remodeling (15).

Alveolar bone is part of the maxillary and mandibular bones which supports the teeth. It is subjected to rapid and continuous remodeling that is associated with tooth eruption and functional demands of mastication. The alveolar bone remodeling ability; is of great importance for positional adaptation of the teeth (16).

Anatomically, the alveolar bone consists of: supporting bone and alveolar bone proper. The alveolar bone proper also known as cribriform plate; contains holes where Volkmann canals pass from the alveolar bone to the PDL. It's also called bundle bone due to the presence of Sharpey's fibers which are inserted into the bone and it is arranged in a parallel orientation corono-apically to the tooth structure. The supporting bone consists of trabecular bone and cortical plate. The cortical bone consists of a layer of compact bone on the lingual and facial surfaces of the alveolar bone. The trabecular bone is found between the cortical bone and the alveolar bone proper (17).

Null hypothesis of this study was that there will be no difference between the control and the study group.

MATERIALS AND METHODS

Study sample

Twenty albino adult male rats whose weight ranges from 200-250 grams (about 6 months of age) were included in this study. The animals were purchased from the Institute of Medical Research, Alexandria University. Rats were housed in special wire mesh cages and supplied with normal, regular diet through the whole experimental period.

The study was conducted after the research ethics committee approval in Faculty of Dentistry, Alexandria University.

Grouping (Randomization technique)

Rats were randomly assigned by (using computer generated random numbers) into two groups:

Group I (control group): 10 rats were injected by vehicle (1 ml phosphate buffer solution) to avoid the effect of any injection stress or buffer-induced effects on the animals.

Group II (glucocorticoid group): 10 rats were injected by methylprednisolone (40 mg/kg) intramuscular for 3 times per week for three weeks (18).

Administration of glucocorticoids

Methyl prednisolone, which was used in this experiment, was purchased from Pfizer Inc. It was available in the form of powder and solvent; they were mixed so that each rat received 40mg/kg.

Euthanization time

All rats were sacrificed after 3 weeks by decapitation. The right molar segments were prepared for light microscopic examination while the left molar segments were prepared for Energy Dispersive X-ray microanalysis (EDX) and Scanning Electron Microscope (SEM). Method of disposal of the rats was done by burning

Histological procedures (19)

Specimens were labeled and fixed in 10% neutral buffered formalin. Afterwards, specimens were placed in 8% tri-chloroacetic acid for decalcification, washed, dehydrated in ascending concentrations of alcohol, cleared with xylene, infiltrated and embedded in paraffin wax blocks. Thin sections of 5 µm thickness were cut using a rotary microtome. Sections were stained with Hematoxylin & Eosin stains (H&E) then examined by light microscope to examine the histological structure of alveolar bone.

Scanning Electron Microscope (SEM)

(20)

Specimens were examined by SEM at the scanning electron microscope unit in the Faculty of Science Alexandria University to study the surface topography of alveolar bone in different groups. The samples were mounted using silver paint on the specimen holder then coated with gold for SEM examination.

Energy Dispersive X-ray (EDX) (20)

Chemical characterization of the samples by analytical X-ray was used to compare the different percentages of calcium and phosphorus in the alveolar bone of the different groups.

Statistical analysis

The data obtained from EDX was collected and analyzed using ANOVA test to compare the overall difference between the three groups

RESULTS

Histological results

Group I (control group)

Results obtained from the control specimens showed normal alveolar bone structure from the crest coronally to the apical part area.

The alveolar bone appeared normal with a continuous layer of active osteoblasts lining the bone surface. Osteocytes were regularly distributed with normal sized lacunae.

The cancellous bone showed thick regular bony trabeculae enclosing normal, cellular and highly vascularized bone marrow spaces lined by flat endosteal cells. (Figure 1)

Group II (glucocorticoid group)

Histological examination of this group from the cervical margin till the apical region showed irregular outline indicating bone resorption and shifting of the crest apically. The bone trabeculae were thin and revealed deeply stained reversal lines.

Osteoblastic cell layer showed a discontinuity along the surface of the alveolar bone in comparison to the control group. Osteocytes were seen with empty or enlarged lacunae containing pyknotic nuclei. Moreover, Osteoclast cells were seen in some regions along the borders occupying the How ship's lacunae.

Cancellous bony trabeculae were irregular and thin enclosing less vascular bone marrow spaces infiltrated with inflammatory cells. (Figure 2)

Scanning electron microscope (SEM) results

Group I (control group)

The buccal cortical plate of the alveolar bone showed a regular smooth surface with a uniform surface topography. It also exhibited multiple nutritive canals with smooth regular borders. (Figure 3)

Group II (glucocorticoid group)

The buccal cortical plate showed pronounced discontinuation of the alveolar bone surface with sever resorption. Generalized roughness with irregular resorptive craters, porosities and deep areas of erosions and pits were observed. (Figure 4)

Energy dispersive x-ray analysis (EDX)

The calcium and phosphorous in different groups were measured by their means and standard deviation. There was statistically significant decrease in calcium level and phosphorous level in glucocorticoid group (group B) in comparison to control group (P1 for calcium<0.001) and (P1 for phosphorous<0.001). (Figure 5) (Table 1)

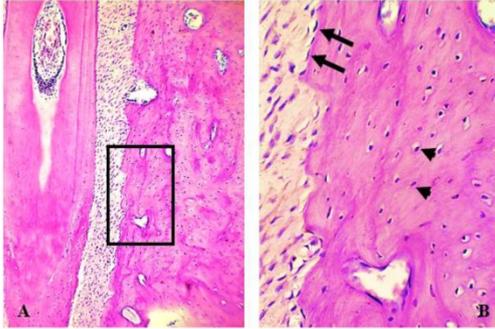


Figure 1: LM (control group I) of the middle region. A: showing regular outline of the alveolar bone. (H&E x100), B: higher magnification showing continuous layer of osteoblasts lining the alveolar bone (arrows), osteocytes are regularly distributed with normal sized lacunae (arrowhead) (H&E x400).

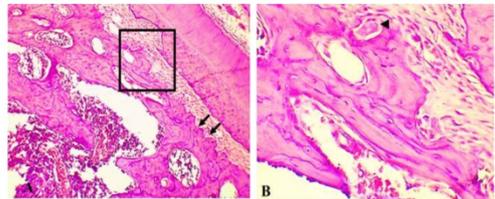


Figure 2: LM of the middle region of the alveolar bone. A: showing irregular resorbed bone surface with deeply stained reversal lines and discontinuity of the osteoblastic cell layer (arrows) (H&E x100). B: higher magnification showing osteoclast in howship's lacunae (arrowhead) and osteocytes with pyknotic nucleus. (H&E x400)

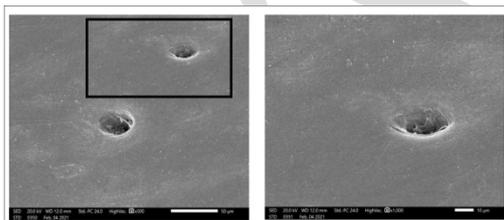


Figure 3: SEM (control group) of the buccal cortical plate. A: showing smooth, regular and uniform outline of the bone surface with regularly outlined nutritive canals (x500). B: SEM of higher magnification of the previous micrograph inset showing smooth bone surface with regular borders of the nutritive canals (x1000).

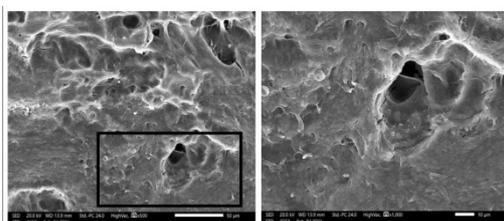


Figure 4: SEM (glucocorticoid group) of the buccal cortical plate. A: showing generalized resorption and porosity. Note the irregular outline with massive abrasion and discontinuity of the cortical plate (x500). B: SEM of higher magnification of the previous inset showing deep resorbed areas and irregularity of the cortical plate (x1000).

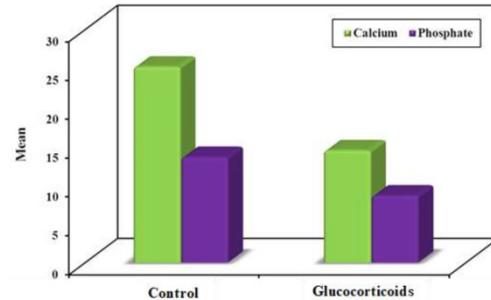


Figure 5: Comparison between the two studied groups according to calcium and phosphate.

Table 1: Comparison between the two studied groups according to calcium and phosphate.

	Calcium	Phosphate	t	p
Control (n=10)				
Min. – Max.	20.74 – 28.96	12.75 – 14.70		
Mean ± SD	25.22 ± 2.73	13.59 ± 0.70	11.886*	<0.001*
Median (IQR)	25.71 (23.95–26.23)	13.65 (12.95–13.83)		
Glucocorticoid (n=10)				
Min. – Max.	12.88 – 16.55	7.51 – 9.59		
Mean ± SD.	14.45 ± 1.45	8.64 ± 0.74	18.982*	<0.001*
Median (IQR)	14.76 (13.10–15.73)	8.88 (7.98–9.22)		

t: Paired t-test

p: p value for comparing between calcium and phosphate in each group

*: Statistically significant at $p \leq 0.05$

DISCUSSION

Glucocorticoids are largely used because of their therapeutic and beneficial in the treatment of autoimmune diseases (21). Despite its clinical benefits, its use is accompanied with many adverse effects as musculoskeletal disorders, glucose intolerance or diabetes mellitus, cataract, skin atrophy, hypertension, an increased risk of infection and delayed wound healing (22, 23).

In addition, glucocorticoids affect calcium as it leads to calcium malabsorption with subsequent secondary hyperparathyroidism due to suppressed expression of intestinal calcium channel (24). Hypocalcemia is a relatively common metabolic abnormality that most commonly occur due to vitamin D deficiency. However, it's accompanied with certain medications as corticosteroids, proton pump inhibitor or antiepileptics and also with other factors as hypomagnesaemia and hypermagnesaemia, metastatic cancer, hyperphosphataemia (12). The present study investigated the biological effect of glucocorticoids when it was orally administrated for 3 weeks on the structure of alveolar bone. This was evaluated by using histological examination, SEM and EDX.

Methyl prednisolone was used in this study and it was administrated orally with dose 40 mg/kg 3 times per week for three weeks. This dose has been shown to affect calcium absorption. This is in accordance with Ericson-Neilsen et al. (2014) (25) and Liu X et al. (2017) (18) who studied the effect of glucocorticoids on calcium level and bone structure.

The results of this study were not in favor of the null hypothesis, where glucocorticoids had a negative effect on calcium level and caused alveolar bone loss. Histological examination showed that the control group had a regular alveolar bone architecture with active osteoblasts lining the bone surface. These results are conforming to Cho et al. (2019) (26) who studied the effect of stem cells on periodontal regeneration and he illustrated the normal structure of alveolar bone.

Nevertheless, glucocorticoid group showed alteration of the normal architecture. Irregular bone surface with multiple osteoclasts lying in How ship's lacunae and signs of bone resorption were evident.

These observations are in accordance with Compston et al. (2018) (27) who studied the adverse effect of GCs on bone and they found that glucocorticoids inhibit bone formation and increase bone resorption. This is mediated by the effect of GC on regulating peroxisome proliferator-activated receptor gamma receptor 2 (PPAR γ 2) and its effect on the Wnt/ β -catenin pathway. Moreover, Sato et al. (2016) (28) illustrated treatment modalities for glucocorticoid-induced osteoporosis and they reported that increased expression of sclerostin results in Wnt/ β -catenin pathway inhibition and diminished osteoblast precursors differentiation

into mature osteoblasts and increased osteocyte and osteoblast apoptosis.

In addition, Lovšin et al. (2021) (29) evaluated the effect of glucocorticoid receptor on RANKL promoter activity and transcription regulation and declared that GC affect bone resorption directly by highly expressing macrophage colony stimulating factor (M-CSF) and RANKL and suppressing osteoprotegerin (OPG) production by osteoblasts and osteocytes, which leads to an increase in the activity and number of mature osteoclasts.

Furthermore, glucocorticoids may have an indirect effect on bone loss as stated by McCloskey et al. (2016) (30) who conducted a meta-analysis of trabecular bone score in fracture risk prediction and they found that GC can lead to increased renal and intestinal calcium losses, diminished production insulin-like growth factor 1 (IGF1) and IGF1 binding protein (IGF-BP). Also, Liu et al. (2013) (31) reviewed the effect of glucocorticoids and suggested management modalities to overcome these adverse effects and proposed that glucocorticoids affect the mineralization of bone matrix by increasing osteoclasts activity and inhibiting calcium absorption in the gut.

Scanning electron microscope (SEM) results supported the histological findings as it showed that the buccal cortical plate of the alveolar bone had a generalized uniform and smooth surface topography. These findings are as stated by De Souza et al. (2018) (32) who studied the normal bone topography by scanning electron microscope and declared the presence of regular and uniform alveolar bone topography in control group.

However, the glucocorticoid group showed generalized roughness of the surface of the buccal cortical plate with severe porosity and deep areas of erosions indicating the progressive bone resorption associated with glucocorticoids. GC excessive use was reported to be associated with osteocytes and osteoblasts apoptosis, hence, bone formation is suppressed due to inhibition of osteoblastic gene transcription and by affecting the viability and function of osteocytes that is important in bone mechanosensory and repair (33).

Energy Dispersive X-Ray was used in this study as it is an effective mean to detect different percentages of calcium and phosphorous in the bone (34, 35). In the current study, the results of the EDX confirmed the SEM observations. Concerning the control group, the calcium level presented the highest values in comparison to glucocorticoid group. While,

phosphorous exhibited the lowest values compared to the study group.

Regarding the EDX results of the glucocorticoid group B, showed a marked decrease in calcium concentration in relation to phosphorous after 3 weeks.

These results are pursuant to Fei, Yurong, et al. (2007) (36) who conducted an elemental analysis on osteoporotic model and stated that "Calcium and phosphorous ratios are reduced in cases of osteoporosis in comparison to control groups. The relative content of calcium in femoral model of osteoporotic groups is less than the control group, while maintaining a strong positive correlation between Ca and P".

To sum up, all the previous light microscopic, SEM and EDX findings in the present study demonstrate that glucocorticoids lead to calcium malabsorption which consequently lead to bone loss.

CONCLUSION

Glucocorticoid induced hypocalcemia is a serious condition which leads to sever bone loss and alteration in the structure of alveolar bone.

Conflict of interest

We declare that we have no conflicts of interest.

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