

Effect of dexamethasone on osteoclast formation in the alveolar bone of rabbits

A. Y. Yasear* and S. A. Hamouda**

Department of Oral Biology, ** Department of Oral Medicine, Faculty of Dentistry
Al-Arab Medical University, Benghazi, Libya

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Abstract

This study was planned to determine whether a short period dexamethasone treatment induce alveolar bone loss or not, therefore, twenty six male albino rabbits were divided into three groups as follow: Test groups I (10 rabbits) received 1.56 mg/kg dexamethasone intraperitoneal injection daily for two weeks, test group II (10 rabbits) received 3.12 mg/kg dexamethasone intraperitoneal injection daily, and the control group (6 rabbits) received saline solution for same period. The animals were sacrificed and histological sections were prepared from the alveolar bone of molar areas of mandible, as well as morphometric analysis of osteoclasts number was performed. The results showed a significant increase in the number of osteoclasts, which indicates that bone loss, is quite inevitable secondary to dexamethasone treatment even in a short period of treatment for two weeks.

Keyword: Dexamethasone, Alveolar bone, Rabbit.

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تأثير الدكساميثازون في تكوين الخلايا الناقضة للعظم في الاسناخ العظمية لأرانب

أكرم يوسف ياسر* و صالح حمودة**

*قسم بايولوجيا الفم، ** قسم طب الفم، كلية طب الفم والأسنان، جامعة العرب الطبية، بنغازي، ليبيا

الخلاصة

لقد تم التخطيط لهذه الدراسة لتبيان هل العلاج بعقار الدكساميثازون لفترة قصيرة ممكن ان يكون له تأثير على إرتشاف العظم أم لا. أستخدم لهذا الغرض ستة و عشرون ارنبا ذكرا. قسمت الارانب الى ثلاثة مجموعات الأولى (عشرة ارانب) حققت بعقار الدكساميثازون بتركيز مقداره ١,٥٦ ملغم /كغم ولمدة اسبوعين واما المجموعة الثانية (عشرة ارانب) فقد تم حقنها بالعقار نفسه لاسبوعين كذلك وبتركيز ٣,١٢ ملغم / كغم وتركت المجموعة الثالثة (سنة ارانب) كمجموعة سيطرة حقنت بالمحلول الملحي المتعادل ولنفس الفترة الزمنية. بعد انتهاء الفترة الزمنية للتجربة تم التضحية بالارانب لاجل عمل مقاطع نسجية من الاسناخ العظمية لاسنان الطواحن الفكية اضافة الى ذلك تم عمل تحليل قياسي احصائي لعدد الخلايا الناقضة للعظم. اظهرت النتائج زيادة مضطردة في عدد الخلايا الناقضة لعظم. ان الزيادة في عدد هذه الخلايا يعنى نقصان المادة العظمية وهذه هي النتيجة الحتمية المصاحبة للعلاج بالدكساميثازون حتى ولو كان لفترة قصيرة تمتد لأسبوعين.

Introduction

Dexamethasone is a long acting glucocorticoid, which is used to treat many inflammatory and autoimmune conditions; also it is given to cancer patients undergoing chemotherapy (1). In dentistry glucocorticoids are known to induce several morphological and biological changes in gingival epithelium (2) and stroma (3) including blood vessels, oral mucosa, periodontal ligament and alveolar bone (4). The role of anti-inflammatory drugs in the bone loss and destructive changes in periodontal diseases has been studied (5-9). These drugs were linked to inhibition of matrix metalloproteinase and prostaglandin expression, which were related to bone remodeling. Glucocorticoids were known to induce bone loss, probably as a result of both direct inhibition of bone formation by osteoblasts (10) and stimulation of bone resorption by osteoclasts (11). Bone resorption reflects the sum of osteoclasts recruitment and death (12,13).

The osteoclast is a member of monocytes/macrophage family that differentiates under the influence of two cytokines namely M-SICSF and receptor activator of NF-B ligand (14,15). Studies that analysed the role of steroidal anti-inflammatory drugs on periodontal tissue are scarce. Thus the aim of the present study is to evaluate the role of systemic use of dexamethasone in the pathogenesis of induced alveolar bone loss in rabbits for a short period of treatment.

Materials and methods

Twenty six apparently healthy male rabbits, were used in the present study. The dose of the drug was calculated according to Nevagi and Kaliwal (16), and then adjusted for the rabbit according to the formula of Pagat and Barnus (17).

The animals were divided into three groups as follows:

- Test group I (ten rabbits): received daily intraperitoneal injection of 1.56 mg/kg dexamethasone (as therapeutic dose) for two weeks.
- Test group II (ten rabbits): received daily intraperitoneal injection of 3.12 mg/kg dexamethasone (as an overdose) for two weeks.
- Control group (6 rabbits): was submitted to daily intraperitoneal injection of normal saline solution for the same period as the test groups.

Following sacrifice, the left and right segments of molar areas of mandible were dissected out and fixed in 10% neutral buffered formalin for 48 hours, then placed in trichloroacetic acids (as decalcifying agent). Following decalcification, the specimens were washed in running water, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Ten serial sections 5

µm thick, were obtained from each rabbit and stained with haematoxylin and eosin. Counting of osteoclasts was conducted in one field from each section. The significance of difference among the three groups was assessed by using one way analysis of variance. The values were expressed as mean ± SD and the level of significant difference between the groups was at $P < 0.05$.

Results

Osteoclasts were identified as large multinucleated cells found at sites where bone was being removed and rest on the bone trabeculae of the alveolar bone (Fig.1). Histological evidence of their resorption activity was provided by their often, being located in a little pits termed Howship's lacuna and because of fixation artifact osteoclasts may be pulled away from the surface on which they were lying during life (Fig. 2). From the table 1, it was that the mean number of cells in therapeutic dose, overdose and control was 14.3, 18 and 1.17, respectively. There were significant differences between the treatment groups and the control one.

Table 1 : Effect of dexamethasone on rabbit osteoclasts

Groups	n	Mean	SD
Therapeutic dose	10	14.3 ^{*a}	2.5
Overdose	10	18.0 [*]	2.8
Control	6	1.2	1.2

* $p < 0.05$ from the control value. ^a $p < 0.05$ from the overdose group

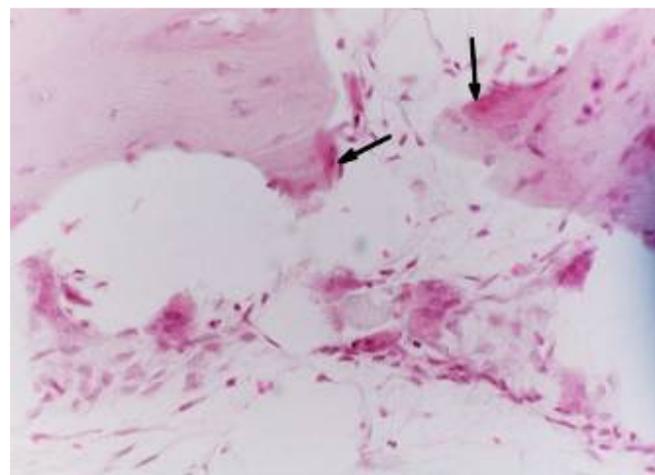


Fig. 1: Molar area of rabbit mandible showing osteoclasts resting on the surface of trabeculae of the spongy bone (arrows). Dexamethasone 1.56 mg/kg. H&E X600.

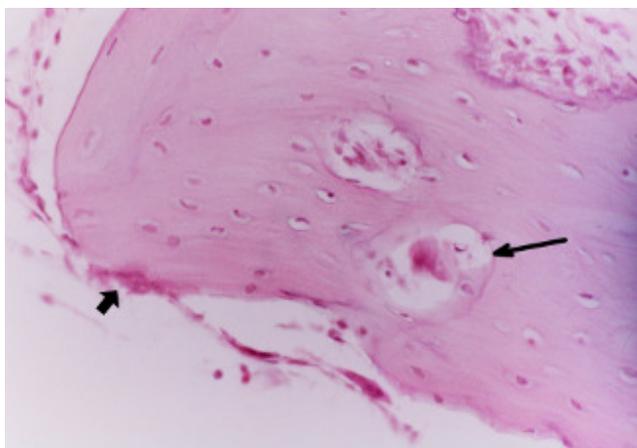


Fig. 2: Molar area of rabbit mandible showing osteoclast (long arrow) separated from the surface of the bone by Howship's lacuna. Thick small arrow is another osteoclast. Dexamethasone 3.12 mg/kg. H&E X 600.

Discussion

The present study showed that different doses of dexamethasone caused an increase in osteoclasts number on the surface of alveolar bone trabeculae, as noted in bone of patients receiving steroid treatment in other study (18). Most of the bone loss occurs during the initial periods of exposure to corticoids, and tends to be dose related (19). The same author has pointed out that bone loss tend to slow down with chronic use but still runs at 3 to 6% annual rate. Our results were consistent with previous finding (20), which reported an increase in osteoclasts of the cancellous part of Swiss Webster mice femur received the same drug. Although the pathogenesis of glucocorticoids mediated bone loss is not completely understood, recent research has provided new insights on the mechanisms of glucocorticoids at cellular and molecular level (12) which was due to direct effects on osteoblasts and osteoclasts. The increased bone resorption by stimulating osteoclastogenesis was related to the increase in the expression of RANK ligand and decreasing of osteoprotegrin (20). In accordance with the increase in bone resorption, glucocorticoids stimulate the expression of collagenase by posttranscriptional mechanisms (15).

Inhibition of bone formation due to decrease in the replication of osteoblasts and their function by preventing

differentiation of osteoblasts into mature functioning cells, and could also be related to the apoptosis of mature osteoblasts (18). The elevated dose of dexamethasone used in this study was chosen in order to better verify the increase in number of osteoclasts compared with the therapeutic dose, and the results indicated a different outcome as compared to that observed with the use of non-steroidal anti-inflammatories (15,18,19). This may add to the knowledge of the biological plausibility of pathogenesis of periodontal diseases, suggesting an association between the use of dexamethasone and increase in alveolar bone loss.

Dexamethasone was acting directly to increase the expression of rank on circulating monocytes which is the osteoclast precursors (22-24). The increase in osteoclast numbers associated with the osteopenia of long term corticosteroid use as primarily the results of an increase in proliferation and differentiation of osteoclasts precursors (24). The increase in efficiency of osteoclast resorption capacity by dexamethasone was related to the ability of the drug to promote and maintain the actin ring of the sealing zone of osteoclasts at the site of resorption (25), which is necessary for normal process of resorption (10).

Excess glucocorticoids reduce both osteoclast and osteoblast precursors (26). Cancellous osteoclast number surprisingly does not decrease as does osteoblast number, presumably due to the promotion of life span of osteoclasts (18,27). It was also an indication of potential interfering of dexamethasone in pathogenesis of bone loss (28).

From the results it can be concluded that the information presented in the article has shown the potential of dexamethasone to be modifier of periodontal breakdown. Thus it should be available to the attention of patient and clinician in clinical approach, that the use of dexamethasone is associated with bone loss even for a short period of treatment.

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