A histomorphometric study of alveolar bone modelling and remodelling in mice fed a boron-deficient diet

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\textbf{ABSTRACT}

Objectives: Emerging evidence indicates that boron (B) plays a role in bone formation and maintenance. Thus, a study was performed to determine whether dietary B-deficiency affects periodontal alveolar bone modelling and remodelling.

Design: Weanling Swiss mice (n = 30) were divided into three groups: control diet (GI, 3 mg B/kg); B-deficient diet (GII, 0.07 mg B/kg); and pair-fed with GII (GIII). The animals were maintained on their respective diets for 9 weeks and then sacrificed. The guidelines of the NIH for the care and use of laboratory animals were observed. The mandibles were resected, fixed, decalcified in 10\% EDTA and embedded in paraffin. Buccolingually oriented sections were obtained at the level of the mesial root of the first lower molar and stained with H–E. Histomorphometric studies were performed separately on the buccal and lingual sides of the periodontal alveolar bone. Percentages of osteoblast surfaces (ObSs), eroded surfaces (ESs), and quiescent surfaces (QSs) were determined.

Results: No statistically significant differences in food intake and body weight were observed between the groups. When compared with GI and GIII mice, GII mice (B-deficient) had 63\% and 48\% reductions in ObS and 58\% and 73\% increases in QS in buccal and lingual plates, respectively. ES were not affected by B nutriture.

Conclusion: The results are evidence that dietary boron deprivation in mice alters periodontal alveolar bone modelling and remodelling by inhibiting bone formation.

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1. Introduction

Alveolar bone is the most malleable of the periodontal tissues, because it is subjected to continuous modelling and remodelling associated with tooth eruption and functional require-
Nutrition is an important modifiable factor in the development and maintenance of bone mass. Dietary components, such as protein, vitamins, and trace elements are required for normal bone metabolism.\textsuperscript{8,9} Emerging evidence indicates that boron (B) plays a role in bone formation and maintenance.\textsuperscript{10–14} To the best of our knowledge, the role of dietary B on alveolar bone modelling and remodelling has not been addressed. Thus, the aim of the present study was to perform a histological and histomorphometric evaluation of periodontal alveolar bone modelling and remodelling under a B-deficient diet in mice.

2. Materials and methods

2.1. Animals

Thirty male weaned (21 d old) Swiss mice were used throughout. They were housed in steel-cages and maintained on a 12:12 h light–dark cycle. All animal experiments were carried out in keeping with the guidelines of the National Institutes of Health for the care and use of laboratory animals (NIH Publication No. 85-23, Rev. 1985). The protocol was examined and approved by the Institutional Ethics Committee of the School of Dentistry, University of Buenos Aires.

2.2. Experimental design

The animals were assigned to 1 of 3 groups, with each group containing 10 animals: control diet (GI, 3 mg B/kg); B-deficient diet (GII, 0.07 mg B/kg); and pair-fed with GII (GIII). The basal diet (Table 1) similar to that used in other studies\textsuperscript{10,15} was based on ground corn that was acid-washed\textsuperscript{16} to reduce its boron content, and vitamin-free casein. It contained adequate amounts of all known essential nutrients plus some mineral elements (e.g., nickel, silicon, vanadium) in nutritional quantities that have been found beneficial to bone health.\textsuperscript{17}

Fresh powder diet and deionized water in plastic cups were provided ad libitum. Body weight and food intake were determined. The animals were maintained on their respective diets for 9 weeks and then sacrificed. The mandibles were resected and fixed in 10% formalin solution.

2.3. Histological processing

The mandibles were decalcified in 10% EDTA and embedded in paraffin. Buccolingually oriented sections were obtained at the level of the mesial root of the first lower molar and stained with hematoxylin–eosin.

2.4. Histological and histomorphometric evaluation

Histological studies and histomorphometric measurements were performed separately on the buccal and lingual sides of the periodontal alveolar bone, which correspond to remodeling and modelling activities, respectively. To clearly define the sides it was necessary to establish the apical limit of the alveolus.\textsuperscript{18,19} Within this context a line a was drawn tangential to the upper cortical of the mandibular canal. Another line was drawn between the uppermost points (A and B) of the buccal and lingual crests. Line CD was drawn so that it bisected the distance between A and B and was perpendicular to line a. In this way the buccal side was limited by points A and D, and the lingual side by points B and D (Fig. 1). The following parameters were determined: percentage of osteoblast surface (ObS), eroded surface (ES), and quiescent surface (QS). Osteoblast surfaces are covered by osteoid seams and mature osteoblasts. Eroded surfaces are scalloped by Howship’s lacunae with or without osteoclasts. Quiescent surfaces are covered by bone lining cells. Histomorphometric evaluation was performed using a microcomputer-based image analysis system (Kontron Elektronik Company, Munich, Germany).

2.5. Statistical analysis

The values for each treatment group were presented as mean ± standard deviation.

The statistical significance of the data was determined using the analysis of variance test (ANOVA). When ANOVA showed a significant difference, the Newman–Keuls multiple-range test was used to define the differences (\(P < 0.01\)) among groups.

3. Results

3.1. Body weight and food intake parameters

No statistically significant differences in food intake and body weight were observed between groups (data not shown).
3.2. Histological and histomorphometric findings

3.2.1. Buccal plates

Light microscopy observation revealed, for GI animals, a predominance (52/69%9%) of bone surface lined by mature cuboidal osteoblasts (ObSs) and bone lining cells (QSs) (48/66%109%). 2/663% of the bone surface was lined by ESs (Figs. 2A and 4A).

In comparison to group I, group II animals exhibited a statistically significant reduction (63%, \(P < 0.01\)) in the percentage of osteoblast surfaces (ObSs, 19% ± 11%) concomitantly with an increase (58%, \(P < 0.01\)) in quiescent surfaces (QSs, 76% ± 10%). No statistically significant differences was observed for eroded surfaces as compared to GI and GIII animals (Figs. 2B and 4A).

Group III animals did not show any statistically significant differences with GI animals for any of the parameters evaluated (Fig. 4B).

None of the groups (I, II, III) exhibited eroded surfaces in the lingual plates.

3.2.2. Lingual plates

The ObS (60% ± 5%) predominated in GI animals. QS comprised 40% ± 7% of the total (Figs. 3A and 4B).

Group II animals exhibited a statistically significant reduction (48%, \(P < 0.01\)) in ObSs (31% ± 7%) and a statistically significant increase (73%, \(P < 0.01\)) in the surfaces lined by bone lining cells (QS, 69% ± 7%) as compared with group I (Figs. 3B and 4B).

Group III animals did not show any statistically significant differences with GI animals for any of the parameters evaluated (Fig. 4B).

None of the groups (I, II, III) exhibited eroded surfaces in the lingual plates.

4. Discussion

The present results provide, for the first time, evidence that the dietary boron (B) deficiency affects the alveolar bone. The histological and histomorphometric analysis evidenced an alteration in periodontal alveolar bone modelling and remodelling in B-deficient mice in terms of a reduction in osteogenic activity concomitantly with an increase in quiescent surfaces. Eroded surfaces were not affected by B nutriture. The differences between the buccal and lingual plates would be due to the differences in behaviour between plates, i.e. remodelling and modelling in the buccal and lingual plates, respectively, as reported previously.18,19 After the eruption period, the relationship between the teeth and their supporting structures remains dynamic, as the former migrate spontaneously within the alveolar process. Teeth migrate mesially in humans and primates but bucco-distally in rodents.1,20,21

Alveolar bone is constantly renewed by modelling and remodelling mechanisms in response to functional demands, local and systemic factors.1,18–22 Nutritional deficiencies in animals have been shown to affect the periodontal tissues.4–7,23–25 In this study, we determined that dietary B deprivation alters periodontal alveolar bone modelling and remodelling by inhibiting bone formation.

Epidemiologic data do not support the suggestion that nutritional deficiencies play an important role in the aetiology and pathogenesis of periodontitis.4 In addition, the efficacy of nutrient supplementation for the therapeutic modulation of the host response in the management of chronic inflammatory periodontal diseases, remains to be determined.7,26,27 One of the most practical applications of nutritional modulation of chronic diseases may be nutrients that regulate the expression of key inflammatory genes.28–30 It has been demonstrated that dietary B supplementation may down-regulate inflammation at a site upstream of cytokine gene activation in the NF-κB regulated pathway.31 Further studies are necessary to evaluate the role of B in the inflammation associated with periodontal disease.

The present study reveals the importance of dietary B in mice periodontal health. The exact cellular and molecular mechanisms by which B deficiency affects alveolar bone remains to be elucidated.

The present findings are consistent with other findings indicating that B deprivation adversely affects bone formation and microstructure. In one study,13 the fourth lumbar vertebrae from male rats exposed to B deprivation (0.1 mg/kg diet) from conception to age 21 weeks were examined by microcomputed tomography and compared to vertebrae from rats fed supplemental B (3 mg/kg diet). Boron deprivation decreased bone volume fraction and trabecular thickness, and
increased trabecular separation and structural model index (a lower value or more plate-like structure is preferable).

Interestingly, B deprivation does not markedly affect the calcium and phosphorus concentrations in bone. Instead, B deprivation affects the concentrations of mineral elements (e.g., magnesium, potassium, copper, zinc)\textsuperscript{10,13} associated with the formation, differentiation and activity of osteoblasts and osteoclasts. The mineral changes in bone, in addition to B deprivation decreasing alveolar bone osteoblast surface in rats\textsuperscript{14} and mice (present study), and chondrocyte density in the growth plate of proliferation of chicks,\textsuperscript{32} suggests that B is beneficial to bone growth and maintenance through affecting

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**Fig. 2 – Microphotograph of the buccal side of the periodontal alveolar bone.** (A) Control diet (GI, 3 mg B/kg) for 9 weeks. Note the bone surface lined by mature osteoblasts. (B) B-deficient diet (GII, 0.07 mg B/kg) for 9 weeks. Note the bone surface lined by bone lining cells (hematoxylin–eosin stain; original magnification 400×).

**Fig. 3 – Microphotograph of the lingual side of the periodontal alveolar bone.** (A) Control diet (GI, 3 mg B/kg) for 9 weeks. Note the bone surface lined by mature osteoblasts. (B) B-deficient diet (GII, 0.07 mg B/kg) for 9 weeks. Note the bone surface lined by bone lining cells (hematoxylin–eosin stain; original magnification 400×).
osteoblast and/or osteoclast presence or activity and not through affecting bone calcium concentration.

In conclusion, our findings suggest that dietary B deprivation in mice alters periodontal alveolar bone modelling and remodelling due to an inhibition of bone formation.

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