Histomorphometric Study of Alveolar Bone Healing in Rats Fed a Boron-Deficient Diet

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ABSTRACT

Bone healing after tooth extraction in rats is a suitable experimental model to study bone formation. Thus, we performed a study to determine the effects of boron (B) deficiency on bone healing by using this model. The first lower right molar of weanling Wistar rats was extracted under anesthesia. The animals were divided into two groups: +B (adequate; 3 mg B/kg diet), and −B (boron-deficient; 0.07 mg/kg diet). The animals in both groups were killed in groups of 10 at 7 and 14 days after surgery. The guidelines of the NIH for the care and use of laboratory animals were observed. The mandibles were resected, fixed, decalcified, and embedded in paraffin. Buccolingually oriented sections were obtained at the level of the mesial alveolus and used for histometric evaluations. Total alveolar volume (TAV) and trabecular bone volume per total volume (BV/TV) in the apical third of the alveolus were determined. Percentages of osteoblast surface (ObS), eroded surface (ES), and quiescent surface (QS) were determined. No statistical significant differences in food intake and body weight were observed. Histomorphometric evaluation found −B rats had 36% and 63% reductions in BV/TV at 7 and 14 days, respectively. When compared with +B rats, −B rats had significant reductions (57% and 87%) in ObS concomitantly with increases (120% and 126%) in QS at 7 and 14 days, respectively. The findings show that boron deficiency results in altered bone healing because of a marked reduction in osteogenesis. Anat Rec, 291:441–447, 2008. © 2008 Wiley-Liss, Inc.

Key words: bone healing; alveolar bone; boron; histomorphometry

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Alveolar bone is a specialized part of the mandibular and maxillary bones that forms the primary support structure for the teeth (Vignery and Baron, 1980; Saffar et al., 1997; Sodek and McKee, 2000). The alveolar bone repair process is initiated immediately after tooth extraction and can be derailed by various endogenous and exogenous factors, e.g., nutrition (Guglielmotti and Cabrini, 1985; Lin et al., 1994; Hsieh et al., 1994; Devlin and Sloan, 2002). 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 (Bonjour, 2005; Prentice et al., 2006; Reid et al., 2006; Heaney, 2007). Emerging evidence indicates that boron (B) plays a role in bone formation and maintenance (Nielsen et al., 1987; Hegsted, et al., 1991; Nielsen and Shuler, 1992; Hunt, 1994; Hunt et al., 1994; Newnham, 1994; Chapin et al., 1998; Armstrong et al., 2000, 2002; Sheng et al., 2001a,b; Devirian and Volpe, 2003; Gallardo-Williams et al., 2003; Nielsen, 2004; Naghii et al., 2006). To the best of our knowledge, this is the first study of the effect of B on bone healing. Thus, a study was performed to determine whether dietary B-deficiency affects alveolar bone healing in rats.

**MATERIALS AND METHODS**

**Animals**

Male Wistar rats (International Laboratory Code Registry: Hsd:Wi-flyb), 21 days old, were used through-

### TABLE 1. Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground corn, acid-washed</td>
<td>713.486</td>
</tr>
<tr>
<td>Casein, vitamin-free</td>
<td>160.000</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>75.000</td>
</tr>
<tr>
<td>Tert-butylhydroquinone</td>
<td>0.014</td>
</tr>
<tr>
<td>dl-α-Tocopherol</td>
<td>0.200</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1.000</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>2.000</td>
</tr>
<tr>
<td>Vitamin mixb</td>
<td>4.000</td>
</tr>
<tr>
<td>Macro mineral mix&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.300</td>
</tr>
<tr>
<td>Trace mineral mix&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15.000</td>
</tr>
<tr>
<td>Total</td>
<td>1000.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Analyzed concentration of boron was about 0.07 mg (9 μmol/kg). To make a 3 mg boron/kg diet, a mix containing 0.0172 g H₃BO₃ and 0.9828 g of dextrose replaced 1.0 g of ground corn in the basal diet.

<sup>b</sup> Composition of the vitamin mix (in mg): vitamin A palmitate (500,000 IU/g), 16; thiamine HCl, 10; pyridoxine HCl, 15; nicotinic acid, 30; dl-pantothenic acid, 48; vitamin B₁₂ (0.1% in mannitol), 50; folic acid, 2; biotin, 1; riboflavin, 27; vitamin K (phyllloquinone), 1; inositol, 50; para aminobenzoic acid, 5; vitamin D₃ (400,000 IU/g), 2.5; and dextrose, 3,742.5.

<sup>c</sup> Composition of the macro mineral mix (in g): CaHPO₄, 17.0; KCl, 7.0; and Mg(C₂H₃O₂)₄H₂O, 5.3.

<sup>d</sup> Composition of the trace element mix (in mg): NaCl, 2,000; Mg(C₂H₃O₂)₂H₂O, 45; CuSO₄5H₂O, 30; Zn(C₂H₃O₂)₂2H₂O, 84; iron powder (dissolved in HCl), 75; NaHAs₄H₂O, 5; KI, 0.4; NaSeO₃, 5H₂O, 1.4; Cr(C₂H₃O₂)₃2H₂O, 2; NH₄VO₃, 0.3; (NH₄)₂MoO₄, 1; NaF, 2; NiCl₂2H₂O, 3.7; NaSiO₂5H₂O, 50, and ground corn (acid-washed), 12,700.2.

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Fig. 1. A: The drawing corresponds to a control alveolus 14 days after the tooth extraction. The zone above line a (hatched area) represents the area considered for the measurement of total alveolar volume (TAV). B: Trabecular bone volume (BV/TV) was measured in the quadrilateral outlined in the zone that corresponds to the apical third of the socket (shaded area). It was determined as follows: Line a: tangent to the upper cortical border of the mandibular canal (A) and perpendicular to the external surface of the buccal plate. B: The point on line a equidistant from A and the external surface on the buccal plate. C: The point on one third of the distance from B to the top of the buccal crest; a line parallel to line a from point C on which distance AB is transported determining point D. 1, mandibular canal.
They were housed in steel cages and maintained on a 12:12 hr light–dark cycle. All animal experiments were carried out according to the guidelines of the National Institutes of Health for the care and use of laboratory animals (NIH Publication Number 85-23, Rev. 1985). The protocol was examined and approved by the institutional ethics committee at the School of Dentistry, University of Buenos Aires.

Experimental Procedure

All animals were anesthetized by intraperitoneal administration of a 4:1 solution of ketamine/xylazine at a dose of 0.15 ml per 100 g of body weight. The first lower right molar of weanling rats was extracted according to the technique described by Guglielmotti and Cabrini (1985). The animals were assigned to two groups: +B (adequate; 3 mg B/kg diet), and −B (boron-deficient; 0.07 mg/kg diet; Table 1). Feeding 3 mg B/kg in the +B group was considered nutritional because it was four times less than the 12 mg B/kg found in commercially prepared rodent diet (Hunt, 1996a). Based on experiments with chicks (Hunt, 1996b) and rats (Bakken and Hunt, 2003; Nielsen, 2004), 3 mg B/kg diet was considered adequate to prevent boron deficiency signs. Fresh food and deionized water in plastic cups were provided ad libitum. Body weight and food intake were determined. The animals in both groups were killed in groups of 10 at 7 and 14 days after surgery. The mandibles were resected and fixed in 10% formalin solution.

Histological Processing

The mandibles were decalcified, embedded in paraffin, and semiserially sectioned, at the level of the mesial alveolus of the first lower right molar, in a frontal plane (bucco-lingual direction) at 10 μm thickness and stained with hematoxylin-eosin.

Histomorphometric Evaluation

Total alveolar volume. Total alveolar volume (TAV) was considered as the bone tissue and its marrow spaces situated above line a drawn tangential to the upper cortical border of the mandibular canal and perpendicular to the external surface of the buccal plate (Fig. 1A).
Trabecular bone volume in the apical third of the alveolus. Trabecular bone volume per total volume (BV/TV, %) was considered as the ratio between the trabecular bone and the total volume, measured in the rectangle ABCD outlined on the apical third of the socket as previously reported (Guglielmotti et al., 1987) and described in the legend to Figure 1B.

The following parameters were determined in the apical third of the alveolus: 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 (Parfitt et al., 1987). Histomorphometric evaluation was performed using a microcomputer-based image analysis system (Kontron Elektronik Company, Munich, Germany).

Statistical Analysis

Student's t-test was used for statistical analysis of the data taking \( \alpha = 0.05 \) and \( \beta = 0.10 \). Data are presented as means \( \pm SD \).

RESULTS

Uncomplicated healing in all rats was observed. No statistical differences in food intake and body weight were observed (data not shown).

Histological Study

Active osteogenesis evidenced by neoformed trabeculae occupying almost the entire alveolus was observed at light microscopy level in +B animals 7 and 14 days after extraction (Figs. 2A, 3A). The woven bone tissue was lined by cuboidal osteoblasts (Fig. 4A). Full epithelialization of the alveolar ridge was observed.

The histologic image 7 days after extraction showed the alveolus occupied by woven bone tissue in the fundus of the socket in −B animals (Fig. 2B). The histologic sections from −B animals killed 14 days postextraction revealed that few trabeculae with scarce bone forming activity were present in the alveolar fundus (Fig. 3B). Trabeculae lined by a predominance of bone lining cells were observed (Fig. 4B). No difference in the healing of soft tissues lining the alveolus was observed compared with +B rats.
Histomorphometric Evaluation

Histomorphometric evaluation found no statistical significant differences in TAV between the groups ($P > 0.05$, data not shown). When compared with +B animals, −B animals had significant reductions (36%, $P < 0.05$ and 63%, $P < 0.01$) in the BV/TV in the apical third of the alveolus at 7 and 14 days, respectively. Statistically significant differences were found between the groups (Fig. 5).

The −B animals showed statistical significant reductions (57% and 87%; $P < 0.01$) in the ObS concomitantly with increases (120% and 126%) in QS at 7 and 14 days, respectively ($P < 0.01$; Fig. 6A,B).

DISCUSSION

The present results provide, for the first time, evidence that the dietary boron (B) deficiency affects the repair of bone tissue. The histological and histomorphometric analysis evidenced an alteration in postextraction bone healing in B-deficient rats in terms of a reduction...
in osteogenic activity concomitantly with an increase in quiescent surfaces. These alterations resulted in a reduction in BV/TV in the apical third of the alveolus in animals fed a B-deficient diet.

Alveolar wound healing provides a suitable model for the study of bone formation in rats and can be considered a sensitive indicator of bone damage under different experimental conditions (Guglielmotti and Cabrini, 1985; Guglielmotti et al., 1985, 1986, 1987; Ubios et al., 1986, 1990, 1991; Lin et al., 1994; Hsieh et al., 1994; Devlin, 2000; Giglio et al., 2000; Tanaka et al., 2001; Kanyama et al., 2003; Elsabeihi and Heersche, 2004; Gorustovich et al., 2004). The accuracy of the methodology used in this experiment has been repeatedly verified both for the procedure of tooth extraction and for the histometric methods. In the first case, an atraumatic surgical technique was used (Guglielmotti and Cabrini, 1985), which allows tooth extraction without postoperative complications. The histometric methods are based on standard stereologic procedures and are used to accurately assess alveolar bone healing affording a numerical characterization of such process (Guglielmotti et al., 1987).

Boron is a bioactive trace element that satisfies several of the criteria for essentiality in humans (Nielsen, 1998, 2000). Considerable evidence has been presented that indicates both nutritional and supranutritional or pharmacologic amounts of B have a beneficial effect on bone formation, composition, and physical characteristics (Nielsen, 2000). For example, dietary supplementation with 50 mg B/kg diet was beneficial to vertebral microarchitecture in rats on strenuous treadmill exercise (Rico et al., 2002). However, like all minerals, B can be toxic when fed in extremely high amounts. Rats fed 500 mg B/kg diet, or over 150 times the +B rats in the present experiment, exhibited depressed weights and femur matrix weight and magnesium and zinc concentrations (Seaborn and Nielsen, 1994). However, the 500 mg B/kg diet did not affect femur calcium and phosphorus concentration or tibia bone density. Thus, supranutritional B intakes in the range of 10 to 20 times nutritional intakes, are also beneficial, but extremely high intakes (greater than 100 times nutritional) may induce some subtle changes in bone. Boron apparently affects bone composition and physical characteristics by influencing the presence or action of hormones involved in bone growth and turnover, and/or through another mechanism that is beneficial to formation or maturation of the organic matrix upon which calcification occurs (Nielsen, 2004).

Recently, it has been demonstrated that B in the form of H$_3$BO$_3$ potently activates the mitogen-activated protein kinase signaling pathway to markedly increase cell proliferation and growth at low concentrations and inhibits these activities at high concentrations (Park et al., 2004). These results are relevant to bone biology given that it has been demonstrated that many of these signaling cascades are required for mesenchymal cell commitment, osteoblast differentiation, and proliferation (Xiao et al., 2000; Miguel et al., 2005; Amaar et al., 2005).

The present study reveals the importance of dietary B in bone healing of the rat mandible. These data do not provide unequivocal evidence of the mechanism by which B deficiency affects bone healing. However, the effects on bone we detected would clearly be due to an inhibition of bone formation. Further studies, therefore, will be necessary to understand the cellular and molecular mechanisms underlying these effects. In conclusion, the findings show that dietary B deficiency results in altered bone healing because of a marked reduction in osteogenesis.

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LITERATURE CITED


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