Stimulation of Bone Formation by Dietary Boron in an Orthopedically Expanded Suture in Rabbits

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ABSTRACT

Objective: To evaluate the effects of dietary boron on bone regeneration in rabbits in response to expansion of the midpalatal suture during different retention periods.

Materials and Methods: Twenty-eight 12-week-old New Zealand white male rabbits were separated into four equal groups: group 1 (B\textsubscript{11001} 10) and group 2 (B\textsubscript{11002} 10) had retention periods of 10 days with or without boron intake, respectively. Group 3 (B\textsubscript{11001} 20, with boron) and group 4 (B\textsubscript{11002} 20, without boron) were retained for 20 days. All groups had a 5-day expansion period. For both B\textsubscript{11001} groups, boron was prepared in distilled water and given to the rabbits during their (1) nursery phase (40 days), (2) expansion phase, and (3) retention period at a dosage of 3 mg/kg daily by oral gavage. Bone regeneration in the midpalatal suture was evaluated by a bone histomorphometric method, and the mineralized area (Md.Ar), fibrosis area (Fb.Ar), mineralized area/fibrosis area (Md.Ar/Fb.Ar), bone area (B.Ar) and osteoblast number (N.Ob) parameters were evaluated.

Results: Statistical analysis showed significant differences between groups for all investigated measurements. Md.Ar ($P < .01$), Md.Ar/Fb.Ar ($P < .001$), B.Ar ($P < .01$), and N.Ob ($P < .01$) parameters were increased and Fb.Ar ($P < .01$) was decreased in groups B\textsubscript{11001} 10 and B\textsubscript{11001} 20. No significant differences were observed during an additional 10-day retention period in all groups ($P < .05$).

Conclusions: Boron has a positive effect on the early phase of bone regeneration of the midpalatal suture in response to expansion and may be beneficial in routine maxillary expansion procedures. (Angle Orthod. 2009;79:984–990.)

KEY WORDS: Boron; Expansion; Histomorphometry; Rabbit

INTRODUCTION

Malocclusion caused by a posterior crossbite or narrow maxillary dental arch width in the maturing patient is commonly treated with rapid maxillary expansion (RME).\textsuperscript{1} This procedure increases the posterior dentition width rapidly, which is followed by active bone formation in the midpalatal suture.\textsuperscript{2}

Although the reason for early relapse is not fully understood, the velocity and quality of bone formation in the midpalatal suture during and after expansion may affect posttreatment relapse.\textsuperscript{1} It might be beneficial, therefore, to accelerate bone formation in the midpalatal suture after expansion to prevent relapse of arch width and to shorten the retention period.\textsuperscript{1,2}

Boron, the fifth element in the periodic table, is the only nonmetal in group 3A elements, but it contains characteristics of both metals and nonmetals. Boron is known to influence a variety of metabolic actions. It interacts with calcium, vitamin D, and magnesium, which are all important in bone metabolism.\textsuperscript{3} Boron accumulates in bone in concentrations that depend on the amount of that element consumed,\textsuperscript{4} and it also has antioxidant properties, which could make it beneficial in preventing atherosclerosis.\textsuperscript{3} Boron is abundant in nature as boric acid and borate and can be obtained in the diet through the consumption of fruits, vegetables, and legumes.\textsuperscript{5} Boron deprivation in animals leads to impaired growth and abnormal bone devel-
opment. The mineral changes in bone, in addition to the fact that its deprivation decreases the alveolar bone surface and activity of osteoblasts in mice, suggests that boron is beneficial to bone growth and maintenance through its effect on the presence or activity of osteoblasts and/or osteoclasts and not by its effect on bone calcium concentration.

In addition to its interaction with other nutrients, supplemental boron as boric acid has been shown to increase bone structure and strength in rats. Wilson and Ruzsler have found that supplemental boron increased long-bone strength in young hens, while McCoy et al. found considerable evidence that both compositional and functional properties of bone were affected positively by boron intake.

There have been few studies attempting to change bone regeneration capacity in the midpalatal suture during maxillary expansion. Sawada and Shimizu investigated the expression of transforming growth factor-β1 (TGF-β1) in RME of the midpalatal suture to evaluate its synergistic effects on bone formation and found that application of TGF-β1 during the early stage was essential to attain the most effective bone formation. Saito and Shimizu evaluated the effects of low-power laser irradiation on bone regeneration during expansion of the midpalatal suture in rats and suggested that laser therapy has therapeutic benefits in inhibiting relapse and shortening the retention period through acceleration of bone regeneration.

The aim of this experimental study was to evaluate the effects of dietary boron on bone regeneration in response to expansion of the midpalatal suture during different retention periods in rabbits. These effects were evaluated with quantitative bone histomorphometric examination. For the purposes of this study, the null hypothesis assumed that boron has no stimulating effects on bone formation in response to expansion of the midpalatal suture during 10- and 20-day retention periods in rabbits.

MATERIALS AND METHODS

Twenty-eight white, male New Zealand rabbits, 12 weeks old and weighing between 2.8 and 3.2 kg, were used. All animals were raised under standardized laboratory conditions of light-and-dark schedule and relative humidity. Pelleted rabbit diet (with normal nutritional levels) and distilled water were provided ad libitum. The experimental protocol was approved by the University of Erciyes Regional Animal Research Ethics Committee. All experimental procedures were performed on anesthetized animals.

The expansion appliance comprised helical springs fabricated from 0.028-inch, stainless steel wires. Helical springs were placed on a grid and activated on a single arm with pliers. The force was measured with a gauge (250 g), and the springs were not reactivated during the experiment. Appliances were placed to the maxillary central incisors of experimental animals under anesthesia. A hole was drilled into each incisor at the level of the lingual gingival papilla, and the springs were inserted into the holes (Figure 1). All groups were subjected to expansion for 5 days. Then the helical springs were removed and a piece of rectangular retainer wire was placed between the two incisors and inserted into the holes for retention (Figure 2). Tooth separation was maintained during the retention phase. The distance between the mesial corners of the incisors was measured at the beginning of the procedure and on the fifth day of expansion with a caliper. Occlusal radiographs were taken at three stages: before expansion, at the end of expansion, and at the end of the retention period (Figure 3).

The animals were randomly separated into four equal groups. Group 1 (B₁00₁₀) and group 2 (B₁00₂₀) had retention periods of 10 days with or without boron intake, respectively. Group 3 (B₁00₁₀, with boron) and group 4 (B₁00₂₀, without boron) were retained for 20 days with the retaining wire after the 5-day expansion period.

The boric acid (99.99% pure; Aldrich Chemical Company, Inc, Milwaukee, Wisc, USA) form of boron (available in powder form) was used in the present study.

Figure 1. Appliance in situ.

Figure 2. Retaining wire placed between rabbit incisors and inserted into incisors’ holes.
research. For the B+ groups, the boron was prepared in distilled water and given to the rabbits during their (40-day) nursery phase and during the expansion and retention periods, at a dosage of 3 mg/kg daily by oral gavage.

The rabbits were monitored during the experiment, and all animals were weighed at the outset, after expansion, and after retention. All animals survived to the end of the study. No wound infection or dehiscence was observed in the animals.

Specimen Preparation

After retention, the rabbits were sacrificed with an overdose of ketamine-xylazine and their premaxillae were dissected and placed in bottles contains 10% formalin. During decalcification, the solution was changed three times a day. After fixation, the retaining wires were removed, and the premaxillae were decalcified with 5% formic acid for 5 days, then fixed again in the same manner and sectioned. The maxillary incisor acted as the primary guide for orienting the sections. The section was cut perpendicular to the sagittal plane and was determined by two points, one at the alveolar crest and the other 4 mm apically. This plane passed through the center of the incisor crown at its gingival portion. The sections were washed, trimmed, and embedded in paraffin. The paraffin blocks were sectioned serially at 5-μm intervals.

Image Acquisition

Histological sections were stained with hematoxylin-eosin prior to optical microscope examination. Measurement for bone histomorphometry was performed 200 μm below the surface of the osseous palate facing the oral cavity because bone formation of the surface area was sometimes irregular and not suitable for quantitative measurement. Measurements were based on observations of the sections under a microscope and calculated using an image analysis program. For this purpose a microscope and digital camera system (Olympus CX41/DP25 Research System, Olympus Corp, Tokyo, Japan) and computer-assisted, image-analysis software (AnalySIS 2.1, Soft-Imaging Software GmbH, Münster, Germany) were used for histomorphometric evaluation.

Histomorphometric Analysis

Histomorphometric evaluation was performed in a blind analysis by two experienced researchers, and the results were an average of the counts. Three histological sections were analyzed for each animal. For data collection, a total area of $9 \times 10^6 \mu m^2$ at a magnification of $4 \times$ was measured (Figure 4). The associated analyzed parameters were the mineralized area (Md.Ar, $\mu m^2$), the fibrosis area (Fb.Ar, $\mu m^2$), the mineralized area/fibrosis area (Md.Ar/Fb.Ar, %), and the bone area (B.Ar, $\mu m^2$) within the premaxillary suture, taken according to descriptions reported by Parfitt et al.9

Immunohistochemistry

Sections were stained with TGF-β2 receptor by the immunohistochemical method, and the number of osteoblasts (Ob.N) reflecting new bone formation was counted.

Statistical Analysis

Statistics were analyzed with the statistical package for social sciences 13.0 (SPSS for Windows, SPSS Inc, Chicago, Ill, USA). Descriptive statistics are given as mean, standard deviation, standard error, minimum, and maximum. Group differences were studied by the Kruskal-Wallis and Mann-Whitney U tests (with the Bonferroni correction). P-values less than .05 were evaluated as statistically significant.
RESULTS

There was no evidence of diarrhea or other gastrointestinal symptoms in any of the animals. The expansion appliance was well tolerated and the animals gained weight. The body weights of two rabbits in the B–10 group decreased during the expansion period, but subsequently recovered. No statistically significant changes in body weight were observed between groups during either the expansion or the retention periods (Table 1).

Biometric analysis for the amount of expansion was done by image analysis software at the most anterior part of the premaxilla on histological sections. Sutural width measurements from histological sections showed that the midpalatal suture had expanded following application of the activated helical loops (springs), with the lateral part of the maxillary bone tipped toward the skull base. It was also determined that the oral side of the suture in the frontal sections had expanded more than the nasal side. The results indicated that the mean amount of expansion was less in the B+20 and B–20 groups (222.10 ± 80.30 μm and 223.97 ± 43.71 μm, respectively) than the B+10 and B–10 groups (246.64 ± 74.99 μm and 236.11 ± 83.38 μm, respectively). However, statistical analysis showed no statistically significant differences (Table 2).

The Kruskal-Wallis test showed statistically significant differences between groups for all investigated histomorphometric parameters. The Md.Ar (P < .01), Fb.Ar (P < .01), Md.Ar/Fb.Ar (P < .001), and B.Ar (P < .01) measurements showed statistically significant differences (Table 3).

According to multiple-comparison analysis, boron supplementation during the 10- and 20-day retention periods resulted in increased Md.Ar (P < .01). For the Md.Ar measurement, the highest value was observed for the B+20 group (41.55 ± 8.07 μm²) and the lowest for the B–10 group (28.51 ± 5.37 μm²) and the difference was found to be statistically significant (P < .01). Significant differences were also noted between groups B+10 and B–10 (P < .05), groups B+10 and B–20 (P < .05), and groups B+20 and B–20 (P < .05).

It was determined that boron supplementation affected the Fb.Ar measurement (P < .01). Statistical tests showed that the Fb.Ar of group B–20 (mean: 20.37 ± 3.83 μm²) was significantly higher than that of group B+10 (mean: 12.25 ± 4.17 μm²) (P < .01) and group B+20 (mean: 14.09 ± 4.49 μm²) (P < .05). No significant changes were observed between groups B+10 and B+20, and between groups B–10 and B–20 (P < .05).

Increased Md.Ar and decreased Fb.Ar measurements in the B+ groups also showed statistically significant higher Md.Ar/Fb.Ar values than the control groups (B–10 and B–20). Significant differences were found between groups B+10 and B–10 (P < .01), B–10 and B+20 (P < .05), and B+20 and B–20 (P < .05).

Bone area was found to be significantly higher in group B+10 (mean: 34.02 ± 6.10 μm²) than in groups B–10 (mean: 25.75 ± 3.43 μm²) (P < .01) and B–20 (mean: 24.71 ± 3.71 μm²) (P < .01). In comparing groups B+20 and B–20, B.Ar measurement was significantly higher in group B+20 (mean: 28.18 ± 4.30 μm²) (P < .05).

Descriptive statistics and multiple comparisons of groups for the N.Ob measurement are presented in Table 4. Statistically significant differences were found

<p>| Table 1. Body Weight Changes (kg) Between Groups During Expansion and Retention Periods* |
|---------------------------------------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean (kg)</th>
<th>SD (kg)</th>
<th>Mean (kg)</th>
<th>SD (kg)</th>
<th>Mean (kg)</th>
<th>SD (kg)</th>
<th>Mean (kg)</th>
<th>SD (kg)</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>B+10</td>
<td>0.363</td>
<td>0.106</td>
<td>0.444</td>
<td>0.124</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td>B–10</td>
<td>0.206</td>
<td>0.254</td>
<td>0.368</td>
<td>0.105</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td>B+20</td>
<td>0.300</td>
<td>0.151</td>
<td>0.350</td>
<td>0.131</td>
<td>0.543</td>
<td>0.214</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>B–20</td>
<td>0.339</td>
<td>0.200</td>
<td>0.400</td>
<td>0.229</td>
<td>0.550</td>
<td>0.262</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
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</table>

* NS indicates not significant; SD, standard deviation; T0, beginning; T1, end of expansion; T2, end of 10-day retention; T3, end of 20-day retention.

<p>| Table 2. Results and Statistical Comparisons of Biometric Analysis for Determination of the Amount of Expansion (μm)* |
|---------------------------------------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Mean (μm)</th>
<th>SD (μm)</th>
<th>Minimum (μm)</th>
<th>Maximum (μm)</th>
<th>ANOVA (F)</th>
<th>Significance</th>
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<tr>
<td>B+10</td>
<td>7</td>
<td>246.64</td>
<td>79.99</td>
<td>147.51</td>
<td>371.93</td>
<td>0.176</td>
<td>NS</td>
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<tr>
<td>B–10</td>
<td>7</td>
<td>236.11</td>
<td>83.38</td>
<td>157.41</td>
<td>391.27</td>
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<tr>
<td>B+20</td>
<td>7</td>
<td>222.10</td>
<td>80.30</td>
<td>152.91</td>
<td>391.27</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>B–20</td>
<td>7</td>
<td>223.97</td>
<td>43.71</td>
<td>155.16</td>
<td>303.12</td>
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</table>

* n indicates sample size; NS, not significant.
in N.Ob between the four groups ($F = 5.373, P = .006$). When multiple comparisons were performed, group B+20, with a mean of $26.64 \pm 6.81$, showed statistically higher N.Ob than both groups B−10 and B−20, whose mean N.Ob measurements were $14.03 \pm 6.74$ and $17.39 \pm 3.21$, respectively.

Thus, according to changes in all histomorphometric parameters, the null hypothesis of this study was rejected.

**DISCUSSION**

Medical researchers have done studies involving the application of different pharmacological agents to increase bone formation. However, little research has been found in the orthodontic literature on stimulating regeneration in the midpalatal suture after expansion. Sawada and Shimizu applied a single dose of TGF-β to the expanding midpalatal suture at an active bone formation site and found that bone formation was significantly stimulated. These authors also evaluated a noninvasive method, that is, low-power Ga-Al-As diode laser irradiation to stimulate an expanding midpalatal suture and found significantly stimulated bone regeneration in the suture during RME in rats. In one study the effects of antioxidants on the early stage of fracture healing were reported; in another, boron was also found to have antioxidant properties. In the current study, we investigated the effects of boron on bone regeneration in response to expansion of the midpalatal suture in rabbits and demonstrated an increase in newly formed mineralized bone area in the suture area with boron supplementation.

Palatal expansion is referred through a multifactorial adaptive response within the midpalatal suture. Mechanical expansion results in distortion of the sutural structure, inducing a biologic chain of events leading to osseous modeling that allows the suture to restore itself to its original architecture. The clinical result is an increase in maxillary skeletal and dentoalveolar width. In the present study, the stimulatory effect of boron treatment on bone regeneration in the midpalatal suture in response to expansion was investigated by using histomorphometry, a reliable technique that is frequently used in the quantitative evaluation of bone remodeling in vivo.

The effects of force on the rate of bone mineralization...
tion can be investigated by animal studies. While the monkey and the cat have maxillary sutures similar in most respects to that of man and have been used in maxillary expansion experiments, the ideal animals with which to obtain a clear picture of bony and sutural changes under stress are the rabbit and the rat.1,3

Burstone and Shafer14 reported that the normal premaxillary suture in young rats measures approximately 20 μm to 60 μm in thickness and found that expansion of the suture by rubber wedges over a period of 5 days resulted in opening the suture to an average width of 377 ± 104 μm. In our study, the premaxillary suture was opened by helical springs. Occlusal radiographs of the rabbits’ snouts showed wide separation of the premaxillary bones after 5 days of expansion, and the sutural widths were found to range between 222.10 μm and 246.64 μm. The amounts of expansion in all groups were similar, showing no statistically significant differences. Sutural width measurements were lower in the long-retention groups, indicating newly forming bone along the medial margins of the bone segments.

Storey et al13 found that, after removal of the helical expansion springs, the bones moved together rapidly, and after 2–3 weeks, the suture appeared to be reconstituted. The authors observed that when separation was maintained for 6 weeks, roentgenograms showed apparent healing, with trabeculae beginning to restore the sutural margins. Thus, at the removal phase of the helical springs, a piece of rectangular retainer wire was placed between the two incisors and inserted into holes in the incisors for retention.

Research has shown that boron may be an essential dietary component for animals and humans.3 It is generally nontoxic, and humans and animals have a homeostatic control for maintaining boron concentration within certain limits.15 Studies on humans have not demonstrated that low boron intake causes developmental abnormalities, but there is evidence that dietary boron benefits human health.15 In the present research, boron supplementation has been shown to affect bone histomorphometric measurements in rabbits. However, the mechanism by which boron treatment promotes bone formation is not fully understood. Mineralized bone area at the midpalatal suture was increased significantly by boron intake. Long-retention groups showed an increase in Md.Ar. The changes from 10 days to 20 days after expansion were similar to the control and there was no statistically significant difference.

Hou et al16 indicated that expansion of the rat suture resulted in ossification of the suture and loss of the normal layered, sutural structure of fibrous tissue sandwiched between the cartilage-covered palatal bone plates. When boron was given to the rabbits during and after expansion, bone formation in the suture was markedly stimulated and Fb.Ar was decreased. These data revealed that mean Fb.Ar was higher than that of animals not given boron during the study period.

Increases in Md.Ar and decreases in Fb.Ar also indicated new bone formation in the boron-supplemented groups. Thus, the Md.Ar/Fb.Ar ratio was increased during the 10-day and 20-day retention periods. Significant changes were also not observed between 10-day to 20-day retention periods in all groups.

Rico et al17 have investigated the effects of boron on bone mineral content and density, trabecular bone volume, and trabecular thickness and found that trabecular bone volume and trabecular thickness were increased by the boron treatment and concluded that boron preserves bone mass in rats. In the present study, B.Ar was also significantly larger in group B+10 than in groups B−10 or B−20. This positive effect of boron on bone area was in accordance with the previous report.

Several studies have shown the biological effects of TGF-β on osteogenic cells. In some in vitro experiments, TGF-β was found to stimulate collagen synthesis, osteopontin, osteonectin and fibronectin production,18 and alkaline phosphatase activity19 in osteoblast-like cells.

TGF-β2 receptor staining was performed by immunohistochemistry to evaluate the osteoblastic activation and count N.Ob in the present study. The expression of TGF-β2 was observed in the midpalatal suture area. TGF-β2 staining increased significantly in group B+20. In the B+10 and B+20 groups, strong TGF-β2 activity was found along the bone edges (the osteoblastic zone of the suture). Related to this increase, the N.Ob was found high (26.64 ± 6.81) for group B+20. A lower N.Ob was counted in groups that had no boron intake (groups B−10 and B−20).

In the current study, systemic boron intake and its effect on bone regeneration was evaluated in response to maxillary expansion during different retention periods. There are some limitations in evaluating the effects of systemically administered pharmacological agents and nutrients on bone formation. To minimize systemically adverse effects and to support bone formation within a definite time interval and in a definite area, it is important to apply it locally. Thus, future investigations will need to employ commercially available boron products that are suitable for local administration. It is also important to evaluate the pure effects of the product on bone regeneration.

CONCLUSION

These findings suggest that dietary boron can stimulate bone regeneration in an orthopedically expanded midpalatal suture during both the expansion and retention periods.
ACKNOWLEDGMENTS

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REFERENCES