Local Delivery of an Amino Bisphosphonate Prevents the Resorptive Phase of Alveolar Bone Following Mucoperiosteal Flap Surgery in Rats

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Mucoperiosteal flaps are used to access the bone and root surface in a wide range of periodontal procedures and in implant surgery. We have demonstrated that the mucoperiosteal surgical flap of the rat mandible produces a transient burst of alveolar bone resorption similar to the clinical observations in humans. This resorptive activity, when coupled with local irritation factors, may cause confined alveolar bone loss. Recently, we have demonstrated that an amino bisphosphonate, which is used in preventing systemic bone resorption in osteoporosis and other bone diseases, reduces alveolar bone resorption in the rat model when administered systemically. In this study we evaluated the effect of local delivery of the amino bisphosphonate on bone resorption associated with mucoperiosteal flaps. Following mucoperiosteal flap elevation in the premolar and molar region of the rat mandible, a surgical pellet soaked with amino bisphosphonate was locally applied on the exposed bone surface and covered by flap. The results show that local delivery of amino bisphosphonate reduces significantly alveolar bone resorption activated by mucoperiosteal flap surgery. This study suggests that local application of amino bisphosphonate can be used as an adjunct in therapy for reducing bone resorption following surgery. J Periodontol 1997;68:884–889.

Key Words: Alveolar bone loss; bone resorption; bone remodeling; osteoclasts; surgical flaps; amino bisphosphonate/therapeutic use; animal studies.

Mucoperiosteal flaps are used to access the bone and root surface for debridement, pocket elimination, management of periodontal defects, and in regeneration procedures, as well as implant surgery. During the dissective procedure, the periosteum is usually separated from the alveolar bone proper, particularly in the area of attached gingiva extending behind the mucogingival junction. A layer of lining cells remains attached to the bone surface, and the rest of the fibrous tissue layer is retained as part of the reflected flap. Many reports show that periodontal surgery stimulates osteoclast activity with varying amounts of alveolar crest loss. In orthopedic surgery, striking remodeling activity occurs adjacent to the site of injury. Frost described this reaction as regional accelerated phenomenon (RAP). The phenomenon is a transient burst of localized remodeling process following surgical wounding of cortical bone. It was suggested that osteoclasts, which resorb bone, and osteoblasts that form new bone at each stage normally do not exist in sufficient numbers to heal the bone following surgery. The RAP effect which was mainly described in long bones recruits these cells. In a previous paper we reported that a similar RAP occurs in alveolar bone adjacent to teeth of rat mandible, following mucoperiosteal flap surgery. Roberts observed similar RAP phenomena in animals following implant surgery. The experimental model of mucoperiosteal flap surgery adjacent to teeth of rat mandibles was used to explore the inhibitory effect of an amino bisphosphonate (ABT) on alveolar bone resorption following surgery. This amino bisphosphonate given systemically has been
Table 1. Comparison of Treated Versus Untreated Mandibles

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± S.E. (mm²)</th>
<th>Student t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23 ± 1.04</td>
<td></td>
</tr>
<tr>
<td>RAP + saline</td>
<td>12.50 ± 0.77</td>
<td>RAP + AB vs. RAP + saline, paired t-test, P &lt; 0.001</td>
</tr>
<tr>
<td>RAP + AB</td>
<td>18.96 ± 1.37</td>
<td>RAP + AB vs. control, P &lt; 0.05</td>
</tr>
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</table>

The mean ± S.E. are in square mm of bone surface from both buccal and lingual areas. The areas were measured between the CEJ and the root apex. Statistical significance was assessed using the Student t-test for control (normal mandibles) and RAP + saline group (control side) or control and RAP + AB (treated side) group. A paired Student t-test was used for RAP + saline and RAP + AB from the same animal.

shown to reduce significantly active bone resorption without interfering with bone formation.12 Bisphosphonates are carbon-substituted pyrophosphate (PCP) analogs that include potent inhibitors of bone resorption which have been effectively used to control osteolysis or reduce bone loss in Paget’s disease, metastatic bone disease, hypercalcemia of malignancy, and osteoporosis.13 The 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid has been approved for the treatment of post-menopausal osteoporosis in many countries.13

Daily treatment with this drug was shown to progressively increase bone mass of normal quality in the total body.12 In fact, in our rat model,12 AB given intravenously significantly reduced alveolar bone resorption in mucoperiosteal flap procedures. Recently it was also reported that systemic treatment with AB12 or another bisphosphonate12 may be beneficial in preventing alveolar bone destruction associated with periodontal disease. In order to avoid systemic use of AB during confined surgery, we explored the effectiveness of topical application (at the surgical site) of AB in reducing bone resorption. We reported that application of AB, soaked in a gauze sponge and applied locally for short duration (10 seconds), was not effective in inhibiting bone resorption.12

We further explored the effectiveness of topical treatment with AB in preventing bone resorption using an absorbent pellet to deliver the drug. Here, we report that local delivery of AB via an absorbent pellet significantly reduces alveolar bone resorption activated by mucoperiosteal surgery.

MATERIALS AND METHODS

Twenty-five Wistar rats were used in this experiment. The right side of the mandible served as the experimental side (RAP+AB) and the left side as a control (RAP+saline). The rats were anesthetized prior to surgery using a mixture of 25 mg/kg body weight of ketamine hydrochloride and 42 mg/kg body weight of xylazine intraperitoneally (IP).

The surgery performed was a mucoperiosteal flap both on the buccal and lingual aspects in the region of pre-

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Figure 2. In this cross-sectioned rat mandible microangiograph, an extensive resorption of alveolar bone especially on its periodontal aspect (arrows) is seen.

molars and molars on both sides of the mandible, two quadrants per rat, as described previously. The flap was elevated using a special small periosteal elevator. Pellets (1 mm diameter) were formed from a sheet of surgical sponges using a rubber dam punch. A pellet was soaked in 0.025 ml of AB solution and applied to the alveolar bone on both buccal and lingual aspects in the experimental side (right, RAP + AB), and the flap was then re-adapted immediately in place without sutures. The solution was prepared by dissolving 20 mg AB in 1 ml of saline. A 1 mm diameter pellet soaked in saline was then applied to the alveolar bone on both buccal and lingual aspects in the control side (left, RAP + saline). The procedure lasted 20 seconds. The pellet remained in situ for at least 2 hours while the anesthesia was effective. The rats were fed a soft diet for 24 hours after surgery to avoid flap displacement and were sacrificed 21 days following the flap procedure. High-resolution x-ray microangiography analysis was performed. The ground sections were 1.5 ± 0.2 mm thick, performed between the premolar and molar region of the mandible in a buccal-lingual direction (4 or 5 sections in each side of the mandible). The x-ray analysis was performed in a mesiodistal direction using Kodak Extaspeed E safety film in a Faxitron series Hewlett Packard System for 5 seconds and 20 KVP.

The high-resolution microangiographs were scanned, image processed, and analyzed using an imaging system and software designed for the dental area. The capturing was performed by a high-resolution scanner specially designed for x-ray film capturing. The microangiographs were scanned at the resolution of 1,200 × 1,200 DPI, and 256 gray levels per pixel.

The software contains a set of well-known image processing functions, including edge enhancement, smoothness, histogram manipulation functions, and specially designed functions such as gray level viewing along a line and absolute difference between two pictures.

Each sample and its control were photographed on the same film and scanned simultaneously in order to eliminate systematic errors in analysis. The samples were evaluated for bone gain/loss in two steps:

1. Each sample histogram was normalized to its control image histogram. The histogram of the control image was corrected by multiplying it with a normalization factor. The factor was calculated by using the local average gray level of the enamel in the sample divided by the control image. Since the enamel remains unchanged in

G6foam. The Upjohn Company, Kalamazoo, MI.

Rosik Systems Ltd., Tel Aviv, Israel.
both the control and sample images, changes in its average gray level related solely to differences in thickness between the images. Samples or control images which revealed severe thickness in homogeneity were rejected.

2. Histogram equalization, of both the sample and its control image, was done in order to stretch the gray level contrast to its maximum.

We also analyzed alveolar bone loss by measuring the total amount of bone on both buccal and lingual aspects in the area between the cemento-enamel junction (CEJ) and the root apex. The amount of bone measured in the untreated (normal) sections was considered as the standard, or 100%, amount of bone. The treated mandible sections (RAP+AB and RAP+saline) were compared to the untreated (normal) control (Table 1). Using a digitizer, we measured the area of bone between the CEJ and the root apex shown on the x-rays. The total areas measured were similar in all groups. Statistical analysis using the Student t-test was performed (Table 1). One-way ANOVA confirmed a significant effect of the mucoperiosteal flap (RAP+saline) on the amount of alveolar bone resorption ($P < 0.001$). The RAP+AB-treated sites demonstrated significantly less bone resorption ($P < 0.001$).

RESULTS

Large areas of extensive bone resorption resulting in alveolar bone loss (RAP) were observed in cross sections 3 weeks after mucoperiosteal flap surgery (Fig. 1 and Table 1). Most of the extensive bone resorption was seen on the periodontal (inner) aspect of alveolar bone (Fig. 2) and the alveolar crest. Figure 3 shows marked resorption, in a representative histological section, 3 weeks following mucoperiosteal flap surgery. Many resorptive lacunae are observed both on inner and outer aspects of the alveolar process. It is also possible to see newly formed bone (see legend to Fig. 3) expected to appear in the initial reparative stage of the RAP phenomenon. The mucoperiosteal surgery (RAP+saline) (Fig. 4, lower row) produced 40% of bone loss relative to normal mandibles (control group). The mean bone surface of the control and RAP was 23 ± 1.04 and 12.50 ± 0.57, respectively (Table 1).

Topical application of the pellet soaked in 0.025 ml of the AB solution (20 mg/ml) demonstrated marked reduction of bone resorption (Table 1 and Fig. 4), maintaining the height of the alveolar crest. The mean bone surface in the topically treated side (RAP+AB) is 18.96 ± 1.37 mm² compared to the contralateral side of the mandible (RAP+saline) (12.50 ± 0.57). Topical application of the amino bisphosphonate prevented loss of alveolar bone by 52% (Table 1). This reduced bone loss is highly significant ($P < 0.001$). Interestingly, RAP+AB treatment retained 90% of the bone as compared to the control group (normal mandibles) (Table 1). Only small areas of bone resorption confined to the coronal 1 mm of the inner aspect of the alveolar bone crest could still be observed. Figure 5 demonstrates higher bone density of the RAP+AB-treated sites (Fig. 5 E) in comparison to RAP+saline sites (Fig. 5 C), using the image processing analysis. In Figure 5, the relative bone density gain/loss is represented by a histogram of radiodensity of similar areas (17,550 pixels) of microradiographic sections.

DISCUSSION

To the best of our knowledge, this is the first report to demonstrate the effectiveness of topical delivery of AB
in preventing bone resorption following mucoperiosteal flap surgery.

The uniqueness of this research model made it possible to demonstrate the effectiveness of the local application of AB. In this model, trauma to the surgical area was created by elevating a mucoperiosteal flap for 20 seconds. This trauma suffices enough to activate local remodeling cycles, thus initiating bone resorption (RAP). The main bone resorption occurred on the inner aspect of the alveolar bone (the periodontal ligament side), the cell-rich zone, that was not directly traumatized by the flap elevation. This observation could suggest a rapid transmission from the traumatic event at the site of injury to neighboring tissues. It is possible that mediators are released and trigger the remodeling cascade in the region. The fact that, in this study, AB that was topically delivered on the outer surface of the alveolar bone reduced the alveolar bone resorption on the periodontal (inner) aspect to the same, and even greater, extent than when administered systemically raises the possibility that AB may cut off the signal from traumatic injury to the alveolar bone. The fine mechanism or mechanisms by which bisphosphonates act on bone resorption are not known; however, they seem to be due either to direct inhibition of osteoclasts, or to an indirect effect on secretion of soluble osteoclast-activating factors by osteoblasts. Various cell types including macrophages and osteoclasts release paracrine factors, including interleukin-6 (IL-6), a multifactorial cytokine, and annexin-II, a family of proteins that bind calcium. These factors interact with phospholipids required for osteoclast recruitment. It was recently proposed that bisphosphonates, in addition to depressing osteoclast resorptive capacity, may depress cell signaling. It seems that this experimental model can be used to explore the mode of action of AB and other bisphosphonates on signal transmission in bone. Systemically delivered AB has already been shown to produce a marked reduction of bone resorption following mucoperiosteal flap surgery in rats, in more than 70% of sections (Fig. 4), maintaining the height of the alveolar crest. Relative to an intravenous dose, the mean oral bioavailability of AB in women is 0.7% for doses ranging from 5 to 40 mg when administered after an overnight fast and 30 minutes before breakfast. Although AB is generally well tolerated, upper gastrointestinal irritation has been reported. The most common adverse effects in descending order (6.6% to 3.1%) are abdominal pain, nausea, dyspepsia, constipation, and diarrhea. Topical delivery overcomes the absorption problems encountered as well as any potential adverse effects on other tissues. The short traumatic injury produced by mucoperiosteal flap surgery can result in total loss of the alveolar bone, if the healing process is disturbed either by infection or trauma, especially if the bone in the area is thin, as in the area of the mesio-buccal root of the maxillary first molar. In our previous study we showed that the affected bone in the rat recovers to its original dimensions after 120 days, if no trauma or infection is present during the healing period. However, both are very common in the oral environment. Evidently, the present study suggests that local treatment of exposed bone by AB delivered in sponges is
able to largely prevent the massive bone resorption activity otherwise occurring.

Multiple actions of the bisphosphonates could be responsible for the inhibitory effect of AB on the alveolar bone resorption following mucogingival flap in the mandible of the rat, both on the outer aspect of the alveolar bone and the inner (periodontal) aspect. The results of this study demonstrate that topical delivery of AB can minimize bone resorption (the RAP process) that is frequently seen during most surgical procedures in dentistry and orthopedics, when periosteum is separated from bone proper. Since the most dramatic therapeutic effects of bisphosphonates depend on the inhibition, not of normal bone remodeling, but of pathologic bone resorption, this research model which mimics pathologic resorptive activity as a result of traumatic injury can be used to explore the intercellular "cross talk" involved in this phenomenon: such studies are currently under investigation in our laboratory.

REFERENCES

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