Effects of bisphosphonates on sutural bone formation and relapse: A histologic and immunohistochemical study

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Introduction: The aim of this experimental study was to evaluate the effects of systemically applied zoledronic acid on bone regeneration in response to expansion of the sagittal suture and relapse in rats. Methods: Thirty-six male Wistar rats were divided into 3 groups. In the first and second groups, saline solution was given subcutaneously after expansion, and the retention periods lasted 14 and 7 days, respectively. In the third group, 0.1 mg of zoledronic acid was diluted with saline solution and given subcutaneously after expansion; the retention period lasted for 7 days. Expansion and relapse amounts were measured by using computed tomography. After the retention period, 6 rats from each group were killed for histologic and immunohistochemical assessments. The other 6 rats from each group were used for observation of the relapse. Results: The histologic evaluation showed that, in groups 1 and 2, the numbers of osteoblasts were less than observed in group 3. When scores of staining intensity were compared, immunoreactivities were statistically significantly increased in group 3 compared with groups 2 and 1. Statistically significant differences were found when the relapse percentages were compared between the groups (P <0.05). The smallest relapse occurred in group 3. Conclusions: Zoledronic acid has positive effects on bone formation in the sagittal suture in response to expansion and decreases the relapse ratio after expansion in rats. (Am J Orthod Dentofacial Orthop 2011;140:e31-e41)

The malocclusions caused by a maxillary transverse deficiency, such as Class I with crowding, Class II with a V-shaped arch, and Class III with a small maxilla in growing patients, are often treated with rapid palatal expansion. After the desired expansion, the suture undergoes remodeling with bone formation, resorption, and fiber rearrangement. This continues until the architectural environment achieves equilibrium. It is well documented that, even after a retention period, the expanded maxillary dental arch has a strong tendency to rebound to its previous form. The literature reported the percentages of relapse after retention from 0% to 45%. Although a 6-month retention period was applied conventionally, Da Silva Filho et al showed that the midpalatal suture was completely ossified from the anterior nasal spine area to the posterior nasal spine area after the retention phase, which was 8 to 9 months postexpansion. It would be potentially beneficial, therefore, to accelerate bone formation in the midpalatal suture after expansion to prevent relapse of the arch width and to shorten the retention period. To maintain the maxillary expansion, stimulation of bone formation in the expanding suture with low-power laser irradiation and transforming growth factor-β1 or vitamin D analog injection has been previously reported. Bisphosphonates are now the most widely used drugs for the treatment of some metabolic bone diseases, such as Paget’s disease, osteogenesis imperfecta, fibrous dysplasia, Gaucher’s disease, malignant hypercalcemia, and osteoporosis. Bisphosphonates can bind to hydroxyapatite crystals in a mineralized bone matrix and make the bone more resistant to osteoclasts, inhibit differentiation of bone marrow precursors into osteoclasts, inhibit osteoclast function by interfering with the mevalonate pathway of cholesterol biosynthesis, and induce apoptosis of osteoclasts. In recent years, it has become known that bisphosphonates not only restrict osteoclastic
activity, but also show osteo-conductive and osteo-inductive effects by increasing osteoblastic activity.15-19

The developments that concern bisphosphonates have received interest in the dentistry field, and bisphosphonates have been used in some dental studies, such as those concerning implant surgery, periodontitis, and alveolar defects. Clinical and radiologic measurements showed that bisphosphonates increase early bone formation rates around dental implants and noticeably increase bone mineral density in the treatment of periodontal defects.20,21 Studies have shown that bisphosphonates can reduce bone loss in animal models of experimentally induced and naturally developing periodontitis.22-24 Also, the effect of bisphosphonate therapy on periodontitis was assessed in human studies.25,26 Six months of treatment with bisphosphonates produced improvement in alveolar bone crest height in patients with type 2 diabetes and established periodontitis.25 Although bisphosphonate therapy improves the outcome of conventional periodontal treatment, as the duration of bisphosphonate use increases, its protective effect appears to decrease.26

Zoledronic acid (ZA), a third-generation, nitrogen-containing heterocyclic imidazole bisphosphonate, has been found to be a more potent inhibitor of bone resorption than other bisphosphonates that are currently available.27 The aim of this study was to evaluate the effects of systemically administered ZA on osteoblastic activity and relapse in the sagittal sutures of rats after expansion. To eliminate the effects of occlusal forces and mastication, this study was designed at the rats’ sagittal sutures.

MATERIAL AND METHODS

Ethical approval (B.30.2.Cum.01.00.00-50/146) was obtained for this study from the Animal Research Ethics Committee at the Comhuriyet University School of Medicine in Sivas, Turkey.

A total of 36 male Wistar rats with a mean weight of 200 ± 10 g were divided into 3 groups of 12 animals each. All animals were kept in separate cages in a 12-hour light and dark environment at a constant temperature of 23°C and fed an ordinary, solid diet and water ad libitum. Body weight was measured every day during the entire experimental period.

Suture expansion was carried out for 7 days on all animals by using an expansion spring made of 0.5-mm diameter stainless steel wire (Dentaurum, Pforzheim, Germany) with 2 helices (Fig 1, A).

The rats were anesthetized with intramuscular injections of a combination of 90 mg per kilogram of ketamine hydrochloride (Ketalar-Eczacibaş, Istanbul, Turkey) and 3 mg per kilogram of xylazine (Rompun-Bayer, Leverkusen, Germany). The hair was shaved, the skin was cleaned, and the area to be operated on was disinfected with povidone-iodine before the surgical procedure (Batticon-Adeka, İstanbul, Turkey). A 1.5 to 2 cm midsagittal incision was made anteroposteriorly through the scalp to expose the sagittal suture. Subsequently, 2 holes were opened symmetrically in the parietal bones with a physiodispenser under saline-solution irrigation. The distance between the 2 holes on opposite sides of the suture was 3 mm. The expansion spring was calibrated in advance to exert an initial expansion force of 120 g. Finally, the expansion spring was placed into the holes, and the scalp was sutured over the spring (Fig 1, B).

After the sutural expansion period, the expansion springs were removed from all rats, and retention appliances were placed into the holes under general anesthesia with the same surgical procedure. Groups 1, 2, and 3 underwent 14, 7, and 7 days of mechanical retention, respectively, with a retention appliance (Fig 1, C). A physiologic saline solution (5 mg/kg, 0.9% sodium chloride) was injected subcutaneously into the animals in groups 1 and 2, which were the control groups. A single dose of 0.1 mg per kilogram of ZA (Zometa, Novartis, East Hanover, NJ) dissolved in 5 mg per kilogram of physiologic saline solution was injected subcutaneously into group 3, which was the study group. To compare the ZA-injection rats with those that underwent identical and longer retention periods, 2 control groups were included. Groups, interventions, and retention periods are shown in Table I.

At the conclusion of the retention period, 6 rats from each group were killed with 200 mg per kilogram of sodium pentobarbital (Pentothal, Abbot, North Chicago, Ill) for histologic and immunohistochemical assessment. The other 6 rats from each group were used for observation of relapse. The retention appliances were removed under general anesthesia, and the rats underwent a 7-day relapse period. At the end of the experimental period, the animals were killed under general anesthesia with 200 mg per kilogram of sodium pentobarbital (Pentothal, Abbot).

The distance between the holes was measured by using computed tomography (CT). The axial CT images of the rats were taken with the Brilliance CT System (Philips Medical Systems, Eindhoven, The Netherlands) in a standard position at Cumhuriyet University, Faculty of Medicine, Department of Radiology, with a tilt of 0°, thickness and table feed of 0.8 mm, and original screen resolution of 512 × 512 matrix with 16 bits. The CT data were transferred directly from the CT scanner to a personal computer as raw data sets without the loss of signals. After the transfer of the CT data to the
personal computer, the distance between the holes was measured with the MX View Workstation program (Philips Medical Systems, Cleveland, Ohio) (Fig 2). CT measurements were taken at the beginning (T1) and the end of the expansion period (T2), after the retention period (T3), and at the end of the follow-up period (T4).

The relapse ratio or the rate of decrease in the distance was calculated according to the following equation: $(T4 - T3) / (T3 - T1) \times 100$.

Cranium samples were dissected and fixed in 10% formalin solution for 24 hours. After fixation, the craniums were decalcified by using ethylenediaminetetraacetic acid (0.1 mol/L, pH = 7.1) solution. During decalcification, the solution was changed every second day at +4°C for 3 weeks. After decalcification, the operation areas were removed from the cranium with a scalpel (Fig 3). Routine paraffin embedding procedures were used. In brief, after decalcification, tissue samples were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin wax; then 5-μm thick sections were cut. All formalin-fixed, paraffin-embedded tissue blocks were evaluated at the Research Laboratory of Histology and Embryology, Celal Bayar University, in Manisa, Turkey. The tissue blocks were chosen carefully after histologic assessment of sections stained with hematoxylin and eosin (Surgipath, 01562E, 01602E, Peterborough, United Kingdom).

For immunohistochemical staining, sections were incubated at 60°C overnight and then held in xylene and rehydrated through a series of ethanol solutions. Sections were washed with distilled water and phosphate-buffered saline solution (PBS; P4417, Sigma-Aldrich, St Louis, Mo) for 10 minutes and then treated with 0.1% trypsin (Zymed, South San Francisco, Calif) at 37°C for 10 minutes and washed with PBS. Sections were delineated with a pen (S2002, Dako, Glostrup, Denmark) and incubated in a solution of 3% hydrogen peroxide (TA-015-HP, Dako) for 5 minutes to inhibit endogenous peroxidase activity. After washing in PBS, the sections were incubated with a nonimmune serum (Ultra V block, cat. No: TA-125-UD, Lab Vision, Fremont, Calif) for 1 hour, and then sections were incubated with primary antibodies: monoclonal mouse anti-osteonectin (33-5500, Zymed), monoclonal mouse anti-osteocalcin (33-5400, Zymed), monoclonal mouse anti-VEGF (SC-7269, Santa Cruz Biotechnology, Inc, Santa Cruz, Calif), polyclonal rabbit anti-TGF-β (SC-146, Santa Cruz Biotechnology, Inc), 1:100 dilution, 1 hour at 4°C in a humidity chamber. The sections were washed 3 times for 5 minutes each with PBS, followed by incubation with biotinylated secondary antibody and then with streptavidin conjugated to horseradish peroxidase in PBS for 30 minutes each (Histostain-plus Peroxidase kit, 85-9043, Zymed). After washing 3 times with PBS, the sections were incubated with di amino benzidine (Dako) for 5 minutes for immunostaining.

After washing with distilled water, the sections were counterstained with Mayer’s hematoxylin (Richard-Allan Scientific, Kalamazoo, Mich) and washed with distilled water. The sections were mounted with a mounting medium and were observed with a BX 40 bright-field microscope (Olympus, Tokyo, Japan). The red-brown precipitate indicated positive findings for the primary antibodies. The

<p>| Table I. Groups, interventions, and retention periods |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Intervention</th>
<th>Retention period (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Expansion + NaCl</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>Expansion + NaCl</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>Expansion + ZA</td>
<td>7</td>
</tr>
</tbody>
</table>

NaCl, Sodium chloride; ZA, zoledronic acid.
negative control samples were processed identically; instead of primary antibodies, the same types of immunoglobulin G were used. Two observers (S.I.), blinded to the clinical information, evaluated the staining scores independently, and no statistical interobserver difference was found. The mean values of the immunohistochemical staining intensities were graded semiquantitatively as mild (+), moderate (++), or strong (+++).

In 40-times magnification, the numbers of active osteoblasts were scored as + (1-10), ++ (11-20), or +++ (≥20). The widths of the vessels were scored as + (narrow), ++ (moderate), or +++ (wide).

**Statistical analysis**

The data were analyzed by using SPSS for Windows (version 13.0, SPSS, Chicago, Ill). The level of significance was set at \( P < 0.05 \). To evaluate the distance measurements in the T1, T2, and T3 periods and compare them between groups, the Kruskal-Wallis test was used. To assess the differences in each group over the various periods, the Friedman and Wilcoxon tests were used.

To evaluate relapse after the retention period (T3-T4), the Kruskal-Wallis test and the Mann-Whitney U test were used.

Histologic and immunohistochemical measurements (T3) were evaluated with the Kruskal-Wallis and Mann-Whitney U tests.

**RESULTS**

Placement of the expansion springs and retention appliances caused temporary reductions in body weight (−5%). Suture separation was successfully achieved with the expansion spring.

There were significant differences between the different time points (T1, T2, T3) on the CT measurements in all groups (\( P < 0.05 \)) for the expansion amounts in the groups. In addition, there were significant differences in the distances between the T1-T2 and T1-T3 periods (\( P < 0.05 \)). However, there was no significant change between T2 and T3 (\( P > 0.05 \)). Table II shows the average measurement and standard deviations at T1, T2, and T3.

There were no significant differences between the groups in the CT measurements at T1. There were no significant differences in the amount of expansion (T1-T2) between the groups. Furthermore, the amount of expansion was maintained after the retention period in all groups (T2-T3) (Table III).

There were significant differences when the relapse percentages between the groups (\( P < 0.05 \)) were compared. The differences in relapse amounts were significant between groups 1 and 2, groups 1 and 3, and groups 2 and 3 (Table III). The smallest relapse percentage was observed in group 3.

The histologic and immunohistochemical appearances of all groups are shown in Figures 4 through 8.
In the examination of the sagittal suture samples stained with hematoxylin and eosin from group 1 (7-day expansion and 14-day retention) under the light microscope, it was observed that (+) active osteoblasts were localized along the suture and the width of the blood vessels (Fig 4). In this group, moderate (+) osteocalcin and osteonectin immunoreactivities were observed in osteoblasts, and minimal/moderate (+) immunoreactivities were observed in suture connective tissues by using an indirect immunohistochemistry method (Figs 5 and 6). In this group, moderate (+) VEGF and TGF-β immunoreactivities were observed in active osteoblasts and suture connective tissues (Figs 7 and 8).

In the examination of the sagittal suture samples stained with hematoxylin and eosin from group 2 (7-day expansion and 7-day retention) under the light microscope, it was observed that (+) active osteoblasts were localized along the suture and the moderate-width blood vessels (Fig 4). In this group, moderate (+) osteocalcin and osteonectin immunoreactivities were observed in osteoblasts, and moderate/strong (+) immunoreactivities were observed in the suture connective tissues (Figs 5 and 6). In this group, moderate (+) to moderate/strong (+) VEGF and TGF-β immunoreactivities were observed in active osteoblasts and suture connective tissues by using an indirect immunohistochemistry method (Figs 7 and 8).

In the examination of the sagittal suture samples stained with hematoxylin and eosin from group 3 (7-day expansion, ZA, and 7-day retention) under the light microscope, it was observed that (++) active osteoblasts were localized along the suture and the width of the blood vessels (Fig 4). In this group, strong (++) osteocalcin and osteonectin immunoreactivities were observed in osteoblasts, and minimal/moderate (+) immunoreactivities were observed in suture connective tissues by using an indirect immunohistochemistry method (Figs 5 and 6). In this group, strong (++) VEGF and TGF-β immunoreactivities were observed in active osteoblasts and suture connective tissues (Figs 7 and 8).

When scores of staining intensity were compared, a statistically significant increase was found in group 3 compared with groups 2 and 1 (Table IV).

**DISCUSSION**

This study demonstrates that ZA-stimulated bone formation and a decreased relapse ratio after expansion in rats’ sagittal sutures. Maxillary expansion is used to correct transversal discrepancies between the maxilla and the mandible. If the retention is not adequate after expansion, a reduction in the width of the expanded maxillary arch might occur. However, after long-term retention is used to prevent relapse, there is generally a reduction in the width of the expanded maxillary arch. Researchers have studied the application of various pharmacologic agents to increase bone formation. However, there is little research in the orthodontic literature on stimulating regeneration in the midpalatal suture after expansion.

Sawada and Shimizu investigated the expression of TGF-β1 in rapid maxillary expansion of the midpalatal suture to evaluate its synergetic effects on bone formation. They found that application of TGF-β1 during the

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**Table II. Comparison of the CT measurements (mm)**

<table>
<thead>
<tr>
<th>Group</th>
<th>T1 Mean ± SD</th>
<th>T2 Mean ± SD</th>
<th>T3 Mean ± SD</th>
<th>Freidman test</th>
<th>Wilcoxon test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 12)</td>
<td>3.00 ± 0.14</td>
<td>5.05 ± 0.31</td>
<td>5.03 ± 0.21</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>2 (n = 12)</td>
<td>2.96 ± 0.13</td>
<td>5.03 ± 0.30</td>
<td>5.02 ± 0.22</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>3 (n = 12)</td>
<td>3.00 ± 0.14</td>
<td>5.1 ± 0.27</td>
<td>5.02 ± 0.20</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Kruskal-Wallis test</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NS, Nonsignificant; *P < 0.05.

**Table III. Relapse ratios**

<table>
<thead>
<tr>
<th>Group 1 (n = 6)</th>
<th>Group 2 (n = 6)</th>
<th>Group 3 (n = 6)</th>
<th>Mann-Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>1–2</td>
</tr>
<tr>
<td>Relapse ratio T3-T4</td>
<td>38.66 ± 5.21</td>
<td>43.00 ± 6.67</td>
<td>13.33 ± 4.26</td>
</tr>
</tbody>
</table>

*P < 0.05.
early stage was essential to obtain the most effective bone formation.

Saito and Shimizu\textsuperscript{10} 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 advantageous benefits in inhibiting relapse and shortening the retention period through acceleration of bone regeneration.

**Fig 4.** Photomicrographs of histologic sections of sutural areas stained with hematoxylin and eosin: A, group 1; B, group 2; C, group 3. ▶ Osteoblast; *blood vessels (400 times magnification).

**Fig 5.** Photomicrographs of immunohistochemical sections of sutural areas stained with anti-osteocalcin antibody: A, group 1; B, group 2; C, group 3. ▶ Osteoblast; S, suture connective tissue (400 times magnification).
Uysal et al.\textsuperscript{12,28} showed that synthetic vitamin D3 analog Ed-71 or dietary boron has positive effects on the early phase of bone regeneration in the midpalatal suture in response to expansion and might be beneficial in routine maxillary expansion procedures.

Bisphosphonates are potent inhibitors of bone resorption. Fleisch\textsuperscript{29} showed that bisphosphonates not only inhibit dissolution of hydroxyapatite crystals, but also affect osteoclast metabolism and function. Lee et al.\textsuperscript{1} investigated the effects of first-generation bisphosphonates

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**Fig 6.** Photomicrographs of immunohistochemical sections of sutural areas stained with anti-osteonectin antibody: \textbf{A}, group 1; \textbf{B}, group 2; \textbf{C}, group 3. ▶ Osteoblast; \textit{S}, suture connective tissue (400 times magnification).

**Fig 7.** Photomicrographs of immunohistochemical sections of sutural areas stained with anti-TGF-β antibody: \textbf{A}, group 1; \textbf{B}, group 2; \textbf{C}, group 3. ▶ Osteoblast, \textit{S}, suture connective tissue (400 times magnification).
etidronate) histochemically and showed that the number of osteoclasts and the relapse ratio were less in the etidronate group than in the control group. They stated that, because the bisphosphonates decrease the number of osteoclasts, the potential for relapse after mechanical expansion of the suture is reduced. Recent studies showed that bisphosphonates not only inhibit osteoblast function but also induce osteoblastic activity. Our study was launched with the intention of preventing relapse not only by decreasing the osteoclasts with bisphosphonates, but also by increasing the amount of new bone formed after expansion in the suture area.

Suture expansion was carried out for 3 days by using an expansion appliance that was calibrated in advance to exert an initial expansion force of 60 g, as in the study by Lee et al. Miyawaki and Forbes investigated the histologic and biochemical responses of the interparietal (sagittal) suture to tensile forces from 50 to 250 g. They stated that suture expansion was achievable with a light force (50-70 g) after 14 experimental periods, but medium (100-150 g) and heavy (200-250 g) forces showed greater sutural opening, more cellular proliferation, and more bone formation. Miyawaki and Forbes found minimal sutural opening at 7 days in the light-force group. To detect visible cellular proliferation and bone formation, we decided to apply a 120-g medium force for 7 days, according to the method of Miyawaki and Forbes and the laboratory experiment we had done before this investigation.

In our study, the histologic evaluation showed that the numbers of osteoblasts and the width of the blood vessels were increased in the ZA-applied group (P <0.05). The immunohistochemical results of our study showed that the immunoreactivities of osteoblasts were higher than in the control groups (P <0.05).

Im et al showed that bisphosphonates are promoters of osteoblast proliferation and maturation. Little et al examined the effect on bone mineral density of a single dose of 3 mg per kilogram of the bisphosphonate pamidronate in distraction osteogenesis. The histologic examination indicated that bisphosphonates increased bone formation. Altundal and Gursoy investigated the influence of bisphosphonates on bone formation after autogenous free-bone grafting in rats and found that bisphosphonates caused a significant increase in the numbers of osteoblasts. Pampu et al evaluated the effects of systemically administered ZA on bone mineral density and bone mineral content at mandibular distraction sites in rabbits. Their results showed significant differences between osteoblast numbers in the experimental group.

In accordance with the literature, as well as with this study, the group to which bisphosphonate was applied after expansion had statistically significant increases in osteoblast numbers and immunoreactivity in the suture region, compared with the other groups.

Experimental evidence has demonstrated that bisphosphonates increase bone mineral density and
regenerate strength in animal models. After these studies, bisphosphonates were used for distraction osteogenesis in humans. Kiely et al.\textsuperscript{31} used bisphosphonates after distraction osteogenesis to increase the regeneration in 7 patients who had deficiency in regenerate formation. In 6 of the 7 patients, this was successful without the need of another intervention.

Lee et al.\textsuperscript{1} found relapse ratios of 25.13% in the control group and 9.60% in the bisphosphonate-applied group after expansion in rats’ sagittal sutures. They suggested that the injection of bisphosphonate after rapid expansion might produce more secure retention by inhibiting bone resorption. In our study, we observed relapse ratios of 43.00% in the control group and 13.33% in the ZA-applied group. Lee et al. found a lower relapse ratio than we did. They administered bisphosphonates daily for a 7-day retention period, whereas we injected a single dose of ZA after expansion. These differences might stem from the duration of bisphosphonate application.

In the initial stage of lower bisphosphonate concentrations, osteoclastic activity is decreased with the balance shifting to more osteoblastic activity, causing increased bone formation. In the later stage, the drug concentrations rise, causing osteoclastic activity to decrease further. This starts to decrease both osteoblastic activity and new capillary formation in new bone, observed as decreased bone turnover and bone repair.\textsuperscript{32} Marini\textsuperscript{31} reported that prolonged high doses of bisphosphonates cause brittle bones that are more susceptible to fractures in children. Histologic evaluation showed that the numbers of osteoblasts and the width of the blood vessel were increased in the single-dose ZA group in our study. A decreased relapse ratio showed that newly formed bone in the ZA group was more resistant than that in the other groups.

In animal studies related to bisphosphonates, it has been determined that bisphosphonates decrease orthodontic tooth movement amounts.\textsuperscript{24-37} Karras et al.\textsuperscript{37} determined that orthodontic tooth movement decreased by 50% to 75% after administration of bisphosphonates in rats. In the literature, 2 articles showed the difficulty of closing the extraction spaces and paralleling the roots in patients who used bisphosphonates.\textsuperscript{38,39} In these articles, only 3 cases were presented.

Since 2003, more cases have been presented that cite the probable relationship between bisphosphonates and osteonecrosis.\textsuperscript{40-43} After the use of bisphosphonates, osteonecrosis has only been seen in the maxilla and the mandible, and not in other bones.\textsuperscript{41,44,45} Anatomic location may be unilateral or bilateral with 68% affecting only the mandible, 28% of cases involving only the maxilla, and 4% both jaws. The reason for this is that the penetration of the medication is much greater in this region as a result of chewing-muscle activity. Also, the bone turnover in this region is 10 times
greater with respect to the tibia. The studies indicated that medications show their effect according to the amount of bisphosphonate used, the length of time it is used, whether it is used regularly, and the type of medication.

It has been stated that, for necrotic bone complaints, the effects can be observed after intravenous ZA or pamidronate use for an average of 9 to 14 months, and after alendronate use for an average of 3 years. Maahs et al determined whether bisphosphonate therapy produces a sufficient condition for jaw osteonecrosis after tooth extraction. Rats were allocated into 3 groups: 11 treated with alendronate, 10 treated with ZA, and 10 controls. The animals were subjected to tooth extractions, and, at the end of bisphosphonate therapy, they were killed. Histologic sections of the surgical sites were processed and analyzed. The authors found that ZA is associated with jaw osteonecrosis, whereas alendronate did not produce a sufficient condition for osteonecrosis after tooth extraction in rats.

Biasotto et al evaluated a novel animal model of bisphosphonate-associated osteonecrosis, which realistically recapitulated the same pathologic human condition. Five Wistar rats were given intravenous ZA of 0.04 mg once a week for 5 weeks. After 2 weeks, the animals had a maxillary molar extracted, producing a 4-mm diameter bone defect at the same site. At 7 weeks after the extraction, the animals were clinically examined, and bone scintigraphy was carried out. After an additional week, the rats were killed, and both CT and histologic analyses were performed. Five rats, not treated with ZA and exposed to the same surgical treatment, were used as controls. At 7 weeks after the extractions, all rats treated with ZA showed expansion of the defect and bone exposure. These features were confirmed by bone scintigraphy. The authors stated that ZA causes osteonecrosis after tooth extraction in rats.

Although there were too many articles that showed a relationship between bisphosphonate and osteonecrosis, in a study presented in 2008 by Malmgren et al, 64 patients were treated for osteogenesis imperfecta with intravenous disodium pamidronate once a month. These patients had an average age of 8.1 years, an average treatment length of 4.5 years, and an average dose of 1623 mg per square meter of pamidronate administered per person. The treatment continued, with 10 patients receiving oral alendronate and 2 receiving intravenous ZA. After an average of 3.6 years, 22 of the 64 patients at an average age of 12.2 years had undergone 38 surgical procedures, mostly for orthodontic purposes, including surgical interventions and tooth extractions. From a clinical standpoint, osteonecrosis was not seen in any of them.

CONCLUSIONS

These findings suggest that systemically administered single-dose bisphosphonates can stimulate initial bone production and decrease the relapse ratio in a short-term relapse period in an orthopedically expanded rat sagittal suture. This suggests the possibility of pharmaceutically assisted retention to maintain the outcome of sutural mechanotherapy in clinical orthodontics. However, further research is needed to answer the following questions.

1. Does short-term bisphosphonate usage stimulate more bone in maxillary expansion in humans?
2. Does the initial increased bone production actually decrease long-term relapse after maxillary suture expansion?
3. Does less tooth movement occur after short-term bisphosphonate usage?

REFERENCES