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Effects of zoledronic acid on sutural bone formation: a computed tomography study

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SUMMARY The aim of this study was to investigate the effects of systemically applied zoledronic acid (ZA) on osteoblastic bone formation and relapse in the rat sagittal suture after expansion.

Eighteen 12-week-old male Wistar rats were divided into three groups. In groups 1 and 2, a saline solution was given subcutaneously after expansion and the retention period lasted for 14 and 7 days, respectively. In group 3, 0.1 mg of ZA was diluted with saline and given subcutaneously after expansion: the retention period lasted for 7 days. Computed tomography (CT) measurements were obtained at the start of the study (T1), after expansion (T2), after the retention period (T3), and after the follow-up period (T4). The amount of expansion and relapse and the density of the newly formed bone in the expansion area were measured. The mean bone density values in hounsfield unit (HU) of the newly formed bone were recorded using MX View Workstation. Data were analysed using the Kruskal–Wallis, Friedman, Wilcoxon, and Mann–Whitney *U*-tests.

The results showed that there were significant differences between the groups in the density of newly formed bone after the retention period ($P < 0.05$). Statistically significant differences were observed when the relapse percentages were compared between the groups ($P < 0.05$). ZA stimulated bone formation and decreased the relapse ratio after expansion in the rat sagittal suture.

Introduction

Bisphosphonates are widely used for the treatment of certain metabolic bone diseases, such as Paget's disease and osteoporosis. They are also used in treating children with osteogenesis imperfecta, fibrous dysplasia, juvenile osteoporosis, and Gaucher's disease (Wedemeyer *et al.*, 2005; Malmgren *et al.*, 2008).

Bisphosphonates bind to hydroxyapatite crystals in mineralized bone matrix and make the bone more resistant to osteoclasts. Bisphosphonates could block the dissolution of hydroxyapatite, inhibit the differentiation of bone marrow precursors into osteoclasts, inhibit osteoclast function by interfering with the mevalonate pathway of cholesterol biosynthesis, and induce apoptosis of osteoclasts (Fisher *et al.*, 1999; Halasy-Nagy *et al.*, 2001; Leite *et al.*, 2006). Zoledronic acid (ZA), which is a third-generation nitrogen-containing heterocyclic imidazole bisphosphonate, is a more potent inhibitor of bone resorption than other bisphosphonates that are currently available (Cheer and Noble, 2001). The increased bone mineral density in patients receiving bisphosphonates has been primarily attributed to the inhibition of osteoclasts and the induction of apoptosis (Reid *et al.*, 2002; Wedemeyer *et al.*, 2005). Im *et al.* (2004) found that ZA has a direct regulating effect on proliferation, differentiation, and gene expression in human osteoblasts. Furthermore, *in vivo* studies have shown enhanced net bone growth into implant porosities and during distraction osteogenesis (DO), as well as a pronounced thickening of periprosthetic cortical bone after bisphosphonate treatment (Little *et al.*, 2003; Wedemeyer *et al.*, 2005).

Maxillary expansion, which is used to correct transverse discrepancies between the maxilla and mandible, occurs through a combination of skeletal and dental expansion. Skeletal expansion involves separating the maxilla at the midpalatal suture, whereas dental expansion results from buccal tipping of the maxillary posterior teeth. Sutural expansion is accomplished by stretching the collagenous fibres, which is then accompanied by new bone formation with associated mitotic figures. After expansion, the suture undergoes remodelling by way of bone formation, resorption, and fibre rearrangement. This continues until the architectural environment achieves equilibrium (Lee *et al.*, 2001). However, although long-term retention is used to prevent relapse, there is generally a reduction of the width of the expanded maxillary arch (Spillane and McNamara, 1995).

To maintain maxillary expansion, stimulation of bone formation in the expanding suture with low-power laser irradiation and transforming growth factor-beta1 or injections of vitamin D analogue has been reported (Sawada and Shimizu, 1996; Saito and Shimizu, 1997; Uysal *et al.*, 2009). The aim of this study was to investigate the effects of

systemically applied ZA on osteoblastic bone formation and relapse in the rat sagittal suture after expansion.

Materials and methods

Ethical approval for the study was obtained from the Animals Research Ethics Committee at Cumhuriyet University School of Medicine (B.30.2.Cum.0.01.00.00-50/146).

A total of 18 12-week-old male Wistar rats with a mean weight of 200 ± 10 g were divided into three groups of six animals each. The rats were kept in separate cages in 12 hour light–dark cycles at a constant temperature of 23°C and fed an ordinary solid diet and water *ad libitum*. Body weight was measured daily during the experimental period.

Sutural expansion was carried out for 7 days in all animals using an expansion spring made of 0.5 mm diameter stainless steel wire (Dentaurum, Pforzheim, Germany) with two helices (Figure 1A). The rats were anaesthetized via an intramuscular injection of a combination of 90 mg/kg ketamine hydrochloride (Ketalar-Eczacıbaşı, Istanbul, Turkey) and 3 mg/kg of xylazine (Rompun-Bayer, Leverkusen, Germany). The hair was shaved, the skin was cleaned, and the area to be operated on was disinfected with povidon-iod (Batticon-Adeka, Istanbul, Turkey) before the surgical procedure. A 1.5–2 cm midsagittal incision was made antero-posteriorly through the scalp to expose the sagittal suture. Subsequently, two holes were opened symmetrically in the parietal bones with a physiodispenser under saline irrigation. The distance between the two 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 (Figure 1B).

At the end of expansion, the spring was removed. Physiological saline (5 mg/kg, 0.9% NaCl) was injected subcutaneously into the animals in groups 1 and 2 (controls). A single dose of 0.1 mg/kg ZA (Zometa; Novartis, East Hanover, New Jersey, USA) dissolved in 5 mg/kg of physiological saline was injected subcutaneously into the animals in group 3 (study group).

After expansion, groups 1, 2, and 3 underwent 14, 7, and 7 days of mechanical retention, respectively, with a retention appliance (Figure 1C). At the end of the retention period, the appliances were removed and the rats were observed for relapse (7 days). At the end of the experimental period, the animals were sacrificed under general anaesthesia with 200 mg/kg sodium pentobarbital (Pentothal; Abbot, North Chicago, Illinois, USA). To compare the ZA-injected rats with those that underwent identical and longer retention periods, two control groups were included. Groups, interventions, and retention periods are shown in Table 1.

The distance between the holes and the density of the newly formed bone were measured using computed tomography (CT). The axial CT images of the rats were taken with the Philips Brilliance CT System (Philips Medical Systems, Eindhoven, Netherlands) in a standard position with a tilt of 0 degrees, a 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 datasets without the loss of signals. After transfer of the CT data, the distance between the holes and the density of the newly formed bone in the expansion area were measured with the MX View Workstation (Philips Medical Systems, Cleveland, Ohio, USA; Figures 2 and 3). CT measurements were taken at the

Table 1 Groups, interventions, and retention periods. ZA, zoledronic acid.

Groups	Intervention	Retention period (days)
1	Expansion + NaCl	14
2	Expansion + NaCl	7
3	Expansion + ZA	7

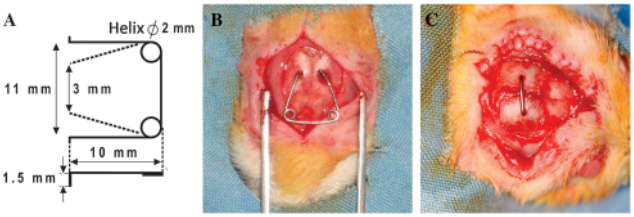

Figure 1 (A) The expansion spring. (B) Insertion of expansion spring. (C) The retention appliance.

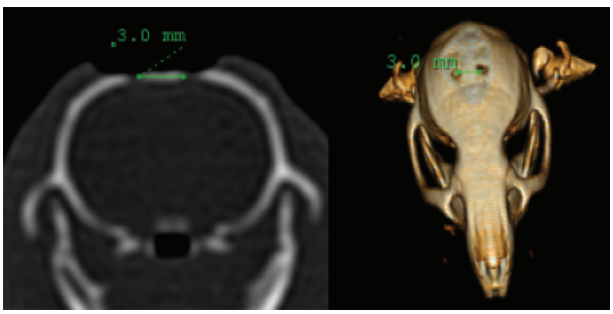

Figure 2 Distance measurement on computed tomography images.

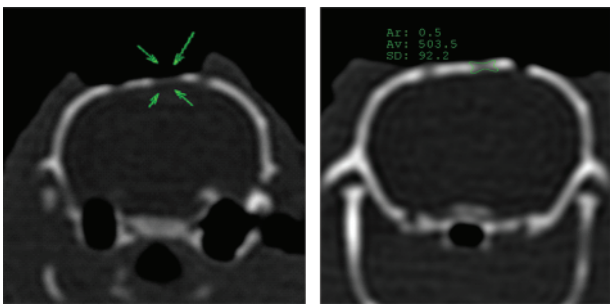

Figure 3 Density measurement of newly formed bone on computed tomography images.

beginning (T1), and end (T2) of expansion, after the retention period (T3), and at the end of the follow-up period (T4).

Statistical analyses

The data were analysed using the Statistical Package for Social Sciences for Windows, version 15.0 (SPSS Inc., Chicago, Illinois, USA). The level of significance was set at $P < 0.05$. To evaluate distance measurements at T1, T2, and T3 and to compare them between groups, a Kruskall–Wallis test was used. To assess the differences within each group over the various periods, Friedman and Wilcoxon tests were used. To compare the density of the newly formed bone (T3) and to evaluate the relapse after the retention period (T3–T4), Kruskal–Wallis and Mann–Whitney *U*-tests were employed.

Results

Clinical evaluation

The insertion of the expansion spring and retention appliances caused a temporary reduction in body weight (−5%). Suture separation was successfully achieved with the expansion spring.

Evaluation of expansion and retention

Within groups. There were significant differences between the different time points (T1, T2, and T3) for the CT measurements in all groups ($P < 0.05$). In addition, there were significant differences in the distances between T1 and T2 and T1 and T3 ($P < 0.05$). However, there was no significant change between T2 and T3 ($P > 0.05$). Table 2 shows the average measurements and standard deviations.

Between groups. There were no significant differences in 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 2).

Evaluation of newly formed bone density in expansion area

The mean densities of newly formed bone were 534.91 ± 20.24, 451.83 ± 13.63, and 595.16 ± 27.47 HU in groups 1, 2, and 3, respectively. There were significant differences between the groups in the density of the newly formed bone at T3 ($P < 0.05$). The differences were statistically significant between groups 1 and 2, 1 and 3, and 2 and 3 (Table 3, Figure 4).

Evaluation of the relapse

There were significant differences when the relapse percentages between the groups ($P < 0.05$) were compared. The differences in the amounts of relapse were significant

Table 2 Comparison of computed tomographic measurements (mm). ZA, zoledronic acid. T1, at the beginning of expansion; T2, at the end of expansion period; T3, after the retention period.

Groups	T1, mean ± SD	T2, mean ± SD	T3, mean ± SD	Wilcoxon			
				Freidman	T1–T2	T1–T3	T2–T3
1 (14 day)	3.02 ± 0.17	5.07 ± 0.42	5.05 ± 0.27	*	*	*	NS
2 (7 day)	2.98 ± 0.27	5.09 ± 0.13	5.08 ± 0.54	*	*	*	NS
3 (7 day, ZA)	3.00 ± 0.61	5.09 ± 0.43	5.07 ± 0.24	*	*	*	NS
Kruskal–Wallis	NS	NS	NS				

NS, not significant. *$P < 0.05$.

Table 3 Measurement of bone density after retention period and relapse ratio. ZA, zoledronic acid. T1, at the beginning of expansion; T2, at the end of expansion period; T3, after the retention period; T4, at the end of the follow-up period.

	Group 1 (14 day), mean ± SD	Group 2 (7 day), mean ± SD	Group 3 (7 day, ZA), mean ± SD	Kruskal–Wallis	Mann–Whitney *U*		
					1–2	1–3	2–3
Hounsfield unit at T3	534.91 ± 20.24	451.83 ± 13.63	595.16 ± 27.47	*	*	*	*
Relapse ratio T3–T4	37.71 ± 5.03	42.75 ± 6.33	12.97 ± 4.75	*	*	*	*

*$P < 0.05$.

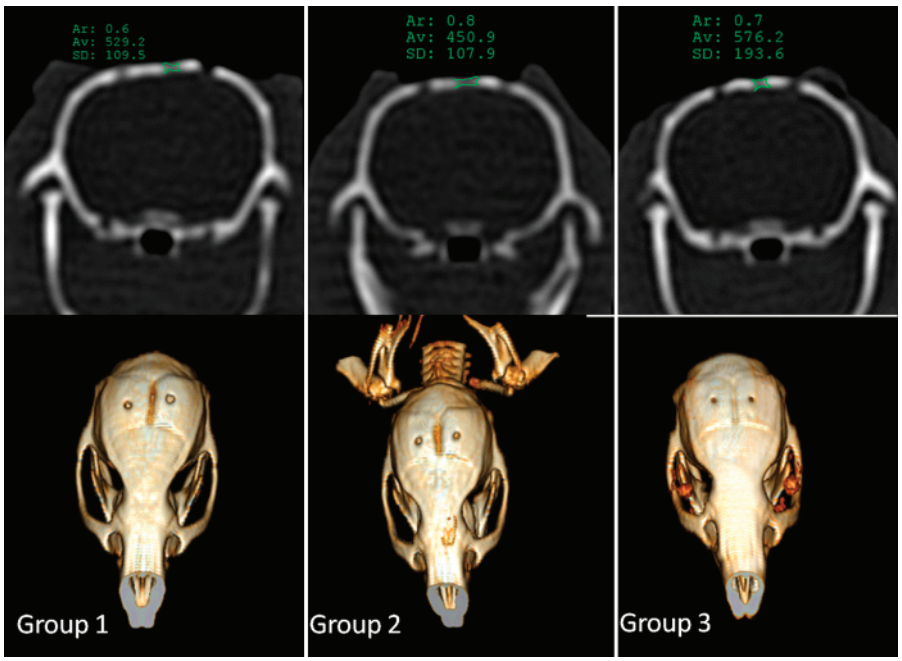

Figure 4 Computed tomography images after the retention period. Note the density of new bone formation in the zoledronic acid applied group (group 3).

between groups 1 and 2, 1 and 3, and 2 and 3 (Table 3). The lowest relapse percentage was observed in group 3.

Discussion

The findings of the study demonstrate that ZA stimulates bone formation and a decrease in relapse ratio after expansion in the rat sagittal suture. Rapid maxillary expansion (RME) is a treatment method for correcting a constricted maxillary dental arch. An extended retention period is necessary to prevent early relapse of the expanded maxilla (Saito and Shimizu, 1997). Sannomiya *et al.* (2007) evaluated new bone formation at the midpalatal suture after surgically assisted RME. The evaluation was performed via optical density analysis, which showed that new bone formation at the midpalatal suture had not been completed after a 3 month period. Da Silva Filho *et al.* (2006) evaluated the midpalatal sutures of children following RME at the end of the retention stage using CT scans. The tomographic images showed that the midpalatal suture was completely ossified from the anterior nasal spine area to the posterior nasal spine at the end of the retention phase, which was 8–9 months post-expansion.

Bisphosphonates are potent inhibitors of bone resorption. Fleisch (2002) showed that bisphosphonates not only inhibit dissolution of hydroxyapatite crystals but also affect osteoclast metabolism and function. Researchers who have investigated the local and systematic effects of bisphosphonates on tooth movement have hypothesized that by decreasing the number of osteoclasts, the rate of orthodontic tooth movement would decrease (Adachi *et al.*, 1994; Igarashi *et al.*, 1994; Liu *et al.*, 2004). In studies related to bisphosphonates, it has been determined that the bisphosphonates decrease the amount of orthodontic tooth movement. Although in some animal studies it was found that bisphosphonates decreased tooth movement, there is no precise information about treatment dose and/or duration of use of bisphosphonates to prevent tooth movement in humans.

Lee *et al.* (2001) investigated the effects of first-generation bisphosphonates (etidronate) histochemically, with reference to the degree of relapse and change in the number of osteoclasts on remodelling of the rat sagittal suture after expansion. The number of osteclasts and relapse ratio were less in the etidronate group than in the control group. Those authors stated that because bisphosphonates decrease the number of osteoclasts, the potential for relapse after mechanical expansion of the suture is reduced. The findings of that study demonstrate the stimulatory effects of ZA on bone regeneration in sutures after expansion using the CT method.

When the densities of new bone formation in the sutures were compared, it was determined, as expected, that bone density volume in group 1 was more than that in group 2. As the retention period increased, the density of the newly formed bone increased. This study was designed with the intention of evaluating relapse not only by decreasing the osteoclasts with bisphosphonate treatment but also increasing the amount of new bone formed after expansion

in the suture area. With this in mind, the formation of new bone density was seen most in the group to which ZA was applied ($P < 0.05$). In the expansion area where ZA was implemented systematically, there was a statistical increase in bone density compared with those animals in the group kept in retention for a longer period of time and the group that was kept in retention for the same duration.

Little *et al.* (2001) evaluated the effects of 1 mg/kg pamidronate, which is the appropriate dose for clinical trials on DO. In that study, bone mineral density was defined using CT. The authors reported that a dose of 1 mg/kg increased bone mineral density and bone mineral content compared with a control group. On the basis of these results, Little *et al.* (2001) stated that pamidronate may have the potential to lead to nephrocalcinosis; thus, new studies are required to determine the effective and accurate dosage. Little *et al.* (2001) also pointed out that as the new generation of bisphosphonates such as ZA exert a stronger influence, with smaller doses, more meaningful results may be produced.

Williams *et al.* (2001), in a rabbit study, compared the effects of two bisphosphonates, 1 mg/kg pamidronate and 0.1 mg/kg ZA, on regenerated bone mineral content in DO using the distraction protocol of Little *et al.* (2001). Evaluation of bone density was conducted with a CT device. ZA was found to be 18 times more potent at producing an increase in bone mineral content than pamidronate. Nephrocalcinosis was identified in 70 per cent of the group that infused with 1 mg/kg pamidronate but not in the livers of either of the two groups infused with ZA.

Pampu *et al.* (2006) evaluated the effects of ZA on mandibular DO in rats with dual energy X-ray absorptiometry (DEXA). In that study, a single dose of 0.1 mg/kg ZA was given intraoperatively. It was found that ZA application led to a significant increase in bone density and bone mineral content in the area of regeneration.

Due to the increased risk of fracture in patients with osteoporosis, Borba *et al.* (2007) systemically administered a single dose of 4 mg of ZA and evaluated bone mineral density with DEXA before and 12 and 18 months after ZA administration. Significant increases in bone mineral density were found at month 12; however, at 18 months, bone mineral density showed a decrease in relation to month 12, but compared with the pre-treatment level, an increase in bone mineral density was still seen.

In accordance with the literature, as in the present study, the group to which bisphosphonate was applied after expansion was found to have a statistically significant increase in bone density in the suture region compared with the other groups. The significant decrease in the amount of relapse in group 3 showed that bisphosphonates can help prevent relapse and can shorten the retention period by stimulating bone regeneration.

Since 2003, there has been an increase in the number of cases presented that mention the probable relationship between the use of bisphosphonate and the formation of osteonecrosis (Wang *et al.*, 2003; Ruggiero *et al.*, 2004; Bagan *et al.*, 2005; Miglioratti *et al.*, 2005; Mavrokokki *et al.*, 2007). All reported cases of bisphosphonate treatment-related osteonecrosis have been in mainly older patients who have a history of malignancy. Therefore, in these cases, in addition to bisphosphonate, many of the patients were treated with steroids, chemotherapy, glucocorticoids, and radiotherapy. These studies state that the use of bisphosphonate is not the only causative factor related to osteonecrosis: the procedures that are implemented may also be a factor (Ruggiero *et al.*, 2004; Leite *et al.*, 2006). Malmgren *et al.* (2008) observed 64 patients (mean age 8.1 years) who were treated for osteogenesis imperfecta with bisphosphonates. Despite long-term intravenous monthly disodiumpamidronate treatment, none of the 64 patients had any clinical signs of osteonecrosis.

Conclusions

The findings of the study demonstrate the stimulatory effects of ZA on bone regeneration in the sagittal suture. By applying ZA after expansion, the retention period can be shortened and the potential for relapse can be reduced. Further studies are required to determine the pharmaceutical aid of bisphosphonates in clinical dentistry.

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