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EXPERIMENTAL STUDY

The effect of zoledronic acid on growth plates and high turnover bones

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Abstract: *Objectives:* Bisphosphonates have preventive effect on bone resorption caused by osteoclasts. We aimed to investigate the histopathological effects of zoledronic acid (ZA) on the jaw and long bones and growth plates of rats.
Methods: Thirty-six 12 week-old female Sprague-Dawley rats were divided into the control (C, n=18) and ZA groups (Z, n=18). Z group animals were administered 0.1 mg/kg saline-diluted ZA intraperitoneally three times per week for 8 weeks. C group animals were administered the same amount of saline simultaneously. At the end of 11th week, half the subjects from either the control group (C1) and ZA group (Z1) were sacrificed. At the end of 14th week, the remaining half from both groups were also sacrificed (C2 and Z2). In all animals, no dental procedures were performed; the posterior and anterior mandible and the knee joint including distal femur and proximal tibia were histopathologically investigated.
Results: Histological examination revealed that inflammation and necrosis were limited to the posterior mandible of the Z1 and Z2 groups, while the anterior mandible and knee joint including distal femur and proximal tibia remained unaffected however the development of the growth plate of the proximal tibia was found to be arrested in animals of the Z1 and Z2groups.
Conclusion: Due to it is inhibitory effect over growth plate and inflammatory and necrotic effect over high turnover bones, zoledronic acid should be administered cautiously, especially in pediatric patients who are still in their growth and development stages *(Fig. 6, Ref. 34)*. Text in PDF *www.elis.sk.*
Key words: inflammation, epiphysis, bisphosphonates.

Bisphosphonates (BPs) are synthetic analogues of pyrophosphate and they have a preventive effect on bone resorption caused by osteoclasts (1). Thus, they elevate bone mineral density and reduce the risk of bone fracture. Due to these effects, BPs are widely used in clinical practice, extending from treatment of metabolic bone diseases and osteoporosis to treatment of primary and metastatic bone tumors (2).

BPs, due to their different structural features, may represent miscellaneous clinical and biological efficacy and they are divided into two groups. The first group involves the non-aminobisphosphonates, i.e., BPs that do not include nitrogen such as clodronate and etidronate. The second group involves the aminobisphosphonates, i.e., BPs that include nitrogen such as zoledronate, pamidronate, ibandronate, alendronate, and risedronate. Zoledronic acid (ZA) belongs to the third generation BPs. BPs have a high affinity to calcium so target to bone tissue. Aminobisphosphonates have higher efficacy than do non-aminobisphosphonates, thus they are more frequently used (3). Zoledronic acid (ZA) is the most efficient BP and is thus most commonly used in clinical practice (4). However, some unexpected side effects of BPs have been reported in clinical practice, particularly for zoledronic acid. One of the most common side effects is osteonecrosis found only in the jawbones (BRONJ, Bifosfonat-Related-Osteonecrosis of the Jaws (5, 6). Although several case reports have suggested that factors such as dental extraction, trauma, and periodontal diseases may act as cofactors in the pathogenesis of BRONJ in patients receiving BPs, this finding is yet to be confirmed (7, 8).

Few experimental and clinical trials have aimed to determine the factors influencing the effects of BPs on jaws and other longer bones. Nevertheless, it has been suggested that bones of the skull and those of the appendicular skeleton give different responses to BPs since they have developed from distinct cell lineages during embryological development (9).

In recent years, the method of distraction osteogenesisis being used in the clinical treatment of many diseases (9) and BPs have been reported to elevate the strength and quality of callus formed due to distraction (10). Some studies have also reported that BPs damage the growth plate and lead to development of shortness by preventing normal bone elongation. However, studies focusing on this topic are limited in number and animal model studies are required (11).

Therefore, we aimed to investigate the effects of ZA, one of the most effective and commonly used BP in clinical practice, on

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the growth plate of developing bones and in the development of BRONJ in a rat model, without taking into consideration the cofactors such as dental extraction, periodontal diseases, and trauma.

Methods

The protocol for this study was approved by the Animal Care and Ethics Committee of our institution. We used thirty-six 12-week-old female Sprague-Dawley rats weighing 220–300 g. All of the animals were housed in separate cages, and were provided with a nutritive diet with free access to food and water. The experiments were performed between 9 am and 5 pm to avoid the effect of diurnal variation. The rats were maintained in this environment for 2 weeks for acclimatization, and were then randomly divided into two groups: control group (C, n=18) and ZA group (Z, n=18). The animals of the Z group were administered 0.1 mg/kg saline-diluted ZA (Zometa; Novartis, Istanbul, Turkey) intraperitoneally three times per week for 8 weeks. The animals of the C group were administered the same amount of saline simultaneously. The dose and time of administration of ZA was determined according to previous studies. All administrations were performed by the same surgeon. At the end of the 11th week, i.e., three weeks after the end of treatment period, half the rats from both the control group (C1) and the ZA (Z1) group were sacrificed by injecting high-dose pentobarbital (Pental; IE Ulagay, Istanbul, Turkey) and at the end of 14th week, the remaining rats in each group, the control group (C2) and ZA (Z2), were sacrificed using the same procedure. In all animals, anesthesia was induced by injecting a mixture of ketamine/xylazine intraperitoneally. After sacrificing the animals, a single surgeon obtained specimens from their jawbones and knee joints, including the distal femur and the proximal tibia. Obtained specimens were fixed with 10 % buffered neutral formalin solution within the next 24 hours. Following fixation, the obtained bone specimens were decalcified using 25 % formic acid solution. The specimens were then subjected to routine follow-up after being embedded in paraffin blocks. Four-µm-thick sections of these blocks were stained using hematoxyllin-eosin (H&E). A complete histopathological evaluation was performed by a single pathologist who was blinded to the groups. Changes in the growth plate of the proximal tibia were also concurrently examined. The density of inflammation was evaluated semi quantitatively. The length of the growth plates were measured using an Olympus DP72 camera (Tokyo, Japan).

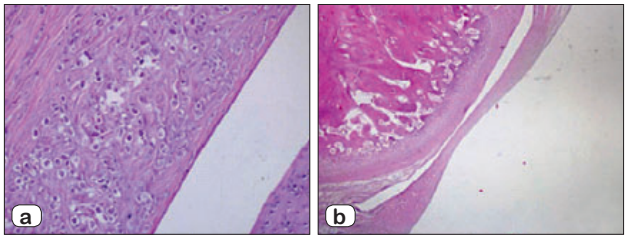

Fig. 1a and b. Mandibular joint surface of an animal from the control group. No inflammation or necrosis is seen in the cartilage tissue (a: H&E, ×40; b: H&E, ×200).

Results

Histopathological investigation involved examination of the specimens obtained from the jaw bone and the knee joint. There was no histopathologically detected inflammation or necrosis in the jaw bones and knee joints in control groups (C1 and C2) (Figs 1a and 1b).

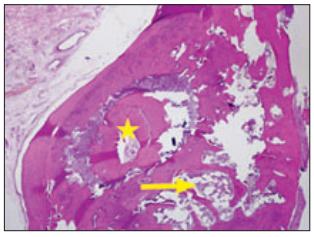

Fig. 2. A mixed-type inflammatory cell infiltration progressing towards the dental root (star) and the alveolar bone (arrow) in the posterior mandible(H&E, ×40).

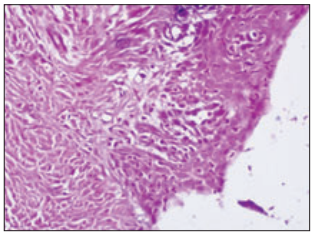

Fig. 3. Inflammatory cells progressing towards the gingival epithelium (H&E, ×400).

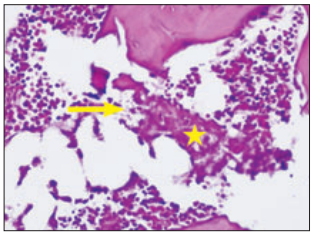

Fig. 4. Necrotic bone spicules in the alveolar bone (star), mixed-type inflammation, and coccoid-type bacteria (H&E, ×400).

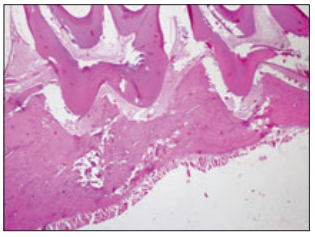

Fig. 5. Absence of inflammation or necrosis at the level of the anterior mandible (H&E, ×40).

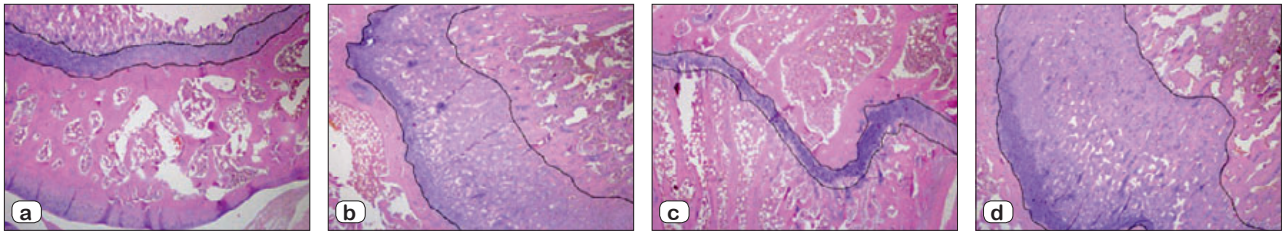

Fig. 6. Difference between the control and study groups with respect to thickness of the growth plates of the proximal tibia. The margins of the growth plates are black (a:C1, 11th week;b:Z1, 11thweek; c:C2, 14th week; d:Z2, 14th week; H&E, ×40).

In Z group (Z1 and Z2) rats, inflammation was observed at the level of the gingival epithelium and along the alveolar bone, resulting in a mixed type of inflammatory cell infiltration (Figs 2 and 3). However, we observed that the inflammation was more pronounced in animals from the Z2 group. We also simultaneously encountered mucosal ulcerations in the jaw bones of animals in the Z1 and Z2 groups. These ulcerations appeared to be more in the Z2 group. Necrosis was also detected in histiological examination of posterior mandibula sections in animals of the Z1 and Z2 groups (Fig. 4).

No inflammation or necrosis was detected at the level of the anterior mandible in animals of both study and control groups (Fig. 5) similarly, in all rats, examination of the specimens obtained from the knee joint revealed no histopathological finding with respect to inflammation or necrosis. However, histopathological investigation of the growth plates in the regions of the proximal tibia showed some differences between the control and study group rats. A discontinuation was found in development of the growth plate of the proximal tibia in the animals of the Z group (Z1 and Z2) (Figs 6a–d). This discontinuation was more pronounced in animals of the Z2 group. Histopathological examination revealed that, alignement of chondrocytes in physis was significantly disturbed in ZA groups (1–2). Height of physis was found to be decreased in ZA groups. Consequently, in the proximal tibia, the thickness of the growth plates of the control and study groups were found to be different.

Discussion

Although the mechanisms of action of BPs on bone metabolism are not yet clear, the data obtained from clinical studies have demonstrated the presence of an association between BPs and osteonecrosis (12). BPs show efficacy on bones when administered in physiological dosages. The BPs that include nitrogen inhibit the action of osteoclasts by blocking farnesyldiphosphate synthase (13, 14). Although their half-life range between 30 minutes and 2 hours within circulation, they can remain in the bones for up to 10 years depending on the skeletal turnover time (13).

Anti-angiogenic effects of BPs are considered to be responsible for the pathophysiology of BRONJ (15). This effect is induced by decreasing the levels of vascular endothelial factor. Thus, BPs reduce the flow and amount of blood circulation within bone tissues (16).

It is not yet known why BP-induced osteonecrosis is observed only in jawbones. Due to the high bone remodeling rate on the surfaces of periodontal and alveolar bones located in the jaw, it has been suggested that high absorption and accumulation of BPs may be responsible for the pathogenesis of the osteonecrosis in jaw bones (17). It has been demonstrated that accumulated BPs have a toxic effect on the oral epithelium besides exerting an osteonecrotic effect. This toxic effect has been reported to cause an infection in the bone structure and facilitate the formation of osteomyelitis (18, 19). In our study, we have observed that osteonecrosis developed only in the jaw bones of animals treated with BPs and that bone structures of the lower extremities were not affected. At the same time, we observed inflammation at the level of the gingival epithelium and along the alveolar bone; this effect was more pronounced in animals of group Z2. In one previous study, mucosal chronic ulceration developed due to soft tissue injury caused by BPs was reported to be the most important factor with respect to the formation of BRONJ (19). In our study, these mucosal ulcerations in jaw bones of rats were detected in both groups Z1 and Z2 additionally we found this effect to be more prominent in the rats of group Z2.

Badros et al (20) and Boonyapakorn et al (21) have reported that inflammation and necrosis scores were much higher for the posterior mandible than for the anterior mandible. The underlying reason for this was found to be higher rates of bone turnover and bone remodeling for the posterior mandible than those for the anterior mandible. Similarly, inflammation and necrosis was found only in the posterior mandible of the animals in our study; no histopathological change was observed in the anterior mandible.

Many studies have focused on the association between the duration of BP administration and the development of BRONJ. Allen and Burr administered high-dose ZA to healthy dogs for 3 years and observed necrosis in their jaw bones (22). In our study, necrosis was detected in the jaw bones of the animals in the study group (Z1 and Z2) following administration of ZA for a relatively short duration.

The necrosis and inflammation scores of the affected jaw bones in the study group animals were found to be different from each other, according to previous studies, this disparity could be due to genetic polymorphism (23) .

Smith et al (24) detected a 3 % reduction in the length of extremities of animals treated with zoledronic acid, and they interpreted this as a result of impaired cellular organization in the growth plate and delayed removal of cartilaginous matrix. Camacho et al (25) reported that BPs may reduce the longitudinal growth rate of the growth plate and that this effect may vary depending on the dosage and duration of the administered medication and age. BPs have a high tropism to the bone matrix and accumulate in this tissue (26). Nitrogen containing BPs are increasingly involved in skeletal pediatric disorders such as osteogenesis imperfeca, juvenile rheumatoid arthritis or pediatric cancers like osteosarcoma (27, 28, 29, 30). Besides its preventive effect on bone resorption the third generation bisphosphonate zoledronic acid has been shown to display an inhibitory effect on bone metastases of various human cancers. There are some recent studies published on this topic indicating the antitumoral effect of ZA on primary tumors and metastases (31).

In their study Battaglia et al reported that use of zoledronic acid had an inhibitory effect on long bones and caused growth arrest (32). There are only a few studies to histologically prove growth plate disturbance due to an effect of ZA on physeal morphology (33). In their study Pataki et al reported that subcutaneous injection of ZA for ten days did not make any significant changes in growth plate thickness, however they found that a significant decrease occurred in the longitudinal growth rate due to ZA treatment (11). In one of the histopathological studies it was reported that amount of cartilaginous matrix in resorptive zone of growth plate was increased due to effect of ZA on growth plate whereas in our study

we did not observe any significant difference between control and ZA groups regarding this issue. In our study, bone volume within both resorptive zone of proximal tibia and metaphysis was found significantly more in ZA groups (1–2) compared to control group whereas in a similar previous study of growth plate, bone volume in resorptive zones of both ZA treated and non-treated groups was found to be comparable (33).

We observed a discontinuation in the development of the growth plate of the proximal tibia in all animals of the study groups (Z1and Z2). This discontinuation was more prominent in group Z2. Consequently, we found growth plate thicknesses of the control and study groups to be different.ZA suppresses matrix metalloproteinase (MMP-9) expression and prevents apoptosis of endothelial cells via vascular endothelial growth factor (VEGF) (34).

We believe that before the clinical use of ZA increases more in pediatric population, the inhibitory effect of this drug on growth plate and its safety should be thoroughly investigated.

As a contribution to literature knowledge, in this study it was found that, one of the most effective BPs in clinical treatment, leads to inflammation and necrosis in the posterior mandible of the jaw bones, but not in other bones of the skeletal system and causes pathological changes in the cellular morphology of the growth plate. In growth plate it delays the removal of cartilaginous matrix leading to discontinuation in the development of the growth plate of the proximal tibia. In our study these histopathological findings were detected following administration of ZA for a relatively short duration when compared to previous studies. Thus, we propose that ZA should be administered cautiously, especially in pediatric patients who are still in their growth and development stages.

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