Evaluation of Effects of Systemic Zoledronic Acid Application on Bone Maturation in the Consolidation Period in Distraction Osteogenesis

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Abstract: Distraction osteogenesis (DO) is a physiological process that generates new bone tissue formation, using progressively separated bone fragments. Recently, several techniques have been investigated to develop the maturation of the new bone tissue. Bisphosphonates was an effective material for the acceleration of bone formation in DO procedures. The purpose of this study was to evaluate the effects of the systemic zoledronic acid application at the beginning of the consolidation period on new bone genesis in a DO model of rat femurs. The rats were divided randomly into 3 groups, as follows: Control group (CNT group) (n = 10), zoledronic acid dosage-1 (n = 10), and dosage-2 (n = 10) groups (ZA-D-1 and ZA-D-2). No treatment was administered in controls, but DO was applied to the rat femurs. A single dose of 0.1 mg/kg and 0.2 mg/kg of zoledronic acid was administered systematically at the beginning of the consolidation period after the distraction in treatment groups, respectively. Histomorphometric analyses were performed on the original distracted bone area and the surrounding bone tissue. Osteoblasts, new bone formation, and fibrosis were scored. New bone formation in the ZA-D-1 and ZA-D-2 groups, when compared with the control group, was detected highly ($P < 0.05$). The numbers of osteoblasts in the ZA-D-1 and ZA-D-2 groups were higher when compared with the controls ($P < 0.05$). Fibrosis in the controls, when compared with the ZA-D-1 and ZA-D-2 groups, was found to be higher ($P < 0.05$). Zoledronic acid application is an effective method for bone maturation in consolidation period in DO.

Key Words: Bisphosphonate, bone, distraction osteogenesis, femur, zoledronic acid

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Distraction osteogenesis (DO) is a technique widely used in dentistry for treating mandibular and maxillar bone and soft tissue deformities, deficiencies, or abnormalities. This technique is a physiological process that generates new bone tissue formation, using progressively separated bone fragments and an external distraction device. Distraction osteogenesis is a favored method of treatment, its principal advantage being that bone tissue formation occurs along with the adaptation of the surrounding soft tissues.[1,2] However, it has some disadvantages because the process requires a long period of consolidation and after this process, the bone resorption can occur.[3,4]

In recent years, several techniques have been investigated to develop the maturation of the new bone tissue (electronic and ultrasonic stimulation, growth factors, hormones, bisphosphonates [BPs], and calcium sulfate). Recently, the authors showed that BP was an effective material for the acceleration of bone formation in DO procedures.[5–9] There are 3 phases in DO, namely the latency, distraction, and consolidation phases. The apparatus-distractor is used over a long term to guarantee the maturation, mineralization, and consolidation of newly formed bone tissue. The distraction apparatus must be kept in the distracted area during this long recovery period, which can lead to social, psychological, and physical burdens. Various materials have been used to shorten the duration of treatment, including mesenchymal stem cells, fibroblast growth factors, bone morphogenetic proteins, prostaglandin E receptor agonists, and bone conduction agents such as calcium sulfate. However, these materials have drawbacks in terms of cost, accessibility, effectiveness, and duration of action. Therefore, new techniques are needed to make DO more efficient.[10]

Bisphosphonates are drugs of carbon-substituted pyrophosphate origin. They are used to prevent and treat increased bone resorption in various skeletal diseases such as Paget disease, osteoporosis, and metastatic bone disease.[11,12]

Bisphosphonates have been reported to have an antiosteoclastic and pro-osteoblastic effect on the bone repair mechanism. Various theories have been suggested, but the exact mechanism of interaction between BPs and bone tissue has not yet been fully elucidated. Previous studies have shown that BPs directly restrict the activation

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of osteoclast cells, can induce mineralized bone formation via the osteoblast cells, and can thus decrease bone loss.[9,13,14]

The purpose of this study was to evaluate the positive effects of the application of systemic zoledronic acid at the beginning of the consolidation period on new bone genesis in a DO model of rat femurs.

MATERIALS AND METHODS

Animals and Study Design

Approval for the experimental design and study was given by Firat University Animal Ethics Committee, Elazig, Turkiye (Date and Protocol Number: 21 February 2017, 188338). Rats were provided by Firat University Experimental Research Center. In total, the experiment was performed on 30 female Sprague Dawley rats aged 4 to 6 months. On the first day of the experiment, the average body weight of the rats was found to be 280 to 300 g. The rats were kept in temperature-controlled cages under a 12-hour light and 12-hour dark cycle, and were provided with easy access to food and water. The number of animals was determined by power analysis in the experiments; 8% deviation, type 1 error (α) 0.05, and type 2 error (β) (power = 0.80), and if the animals were divided into groups, at least 7 animals in each group should be determined. Ten rats per group were included in the study due to the probability of some subjects dying during the experimental period.

First, the rats were divided randomly into 3 groups, as follows:

Control group (CNT group): No treatment was administered, but DO was applied to the rat femurs.

Zoledronic acid dosage-1 group (ZA-D-1 group): A single dose of 0.1 mg/kg of zoledronic acid was administered systematically, according to a previous study, at the beginning of the consolidation period after the distraction.[15]

Zoledronic acid dosage-2 group (ZA-D-2 group): A single dose of 0.2 mg/kg of zoledronic acid was administered systematically, according to a previous study, at the beginning of the consolidation period after the distraction.[15]

The distractor was created using an orthodontic jack embedded in acrylic resin. The distractor was stabilized to the femur with four titanium bone screws. It was set up so that, at the end of each rotation (180 degrees) after each activation of the distractor, it would create a separation of 0.175 mm in the osteotomized bone fragments.

Surgical and Experimental Procedures

General anesthesia was performed by intramuscular administration of 35 mg/kg ketamine hydrochloride and 5 mg/kg xylazine. Surgical operations were performed in full compliance with sterile conditions. After anesthesia, the femoral skin was washed with povidone-iodine before surgery. The operation area was then shaved so as not to hinder the surgical procedure. A 2 cm-long incision was made, avoiding soft tissue damage. Subcutaneous tissues were then carefully exposed. After surgical procedures, the flap and periosteum were removed using a periosteal riser, and bone tissue was accessed. The femoral skin was sutured using 4/0 polyglactin absorbable sutures. Penicillin (40 mg/kg) and an analgesic (tramadol hydrochloride 0.1 mg/kg) were intramuscularly administered to all animals for 3 days after surgery.

Distraction devices were placed in the right femoral bone of the experimental groups in order to standardize the experiment during surgical procedures. Vertical osteotomy of the femur was performed using fissure steel burs. After bicortical holes were drilled in both parts of the femur, the distractor was fixed with titanium bone screws. During the osteotomy, sterile saline solution was used to prevent overheating (Fig. 1 A,B).[16]

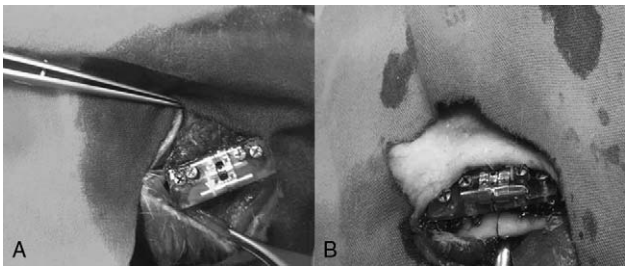

FIGURE 1. (A) Application of the distractor on rat femur bone. (B) After the vertical bone osteotomy.

Distraction was performed with a 5-day latency phase postoperatively, and the distraction process was completed in 10 days. The distractor was activated twice a day in the distraction phase. A 0.175 mm distraction was performed every 12 hours. Animals were euthanized after a 4-week consolidation phase to preserve regenerated bone tissue. During the experimental period, 3 rats in each group died.[16]

Histological Analysis

Histomorphometric analyses were performed on the original distracted bone area and the surrounding bone tissue. The samples were kept in 10% formaldehyde for 72 hours and demineralized in 10% formic acid. They were then dehydrated and embedded in paraffin wax. Hematoxylin-eosin staining and segmentation for microscopic analysis were performed. Sections with a thickness of 6 μm, corresponding to the bone distraction area, were evaluated using a light microscope. The scoring of osteoblasts was done according to the following protocol: osteoblast cells are absent = 0, osteoblasts are slightly visible = 1, osteoblasts are sparsely seen = 2, osteoblasts are densely seen = 3.[12] New bone formation (NBF) was scored as follows: no NBF = 0, slight visible bone formation = 1, moderate visible bone formation = 2, dense visible bone formation = 3.[12] Fibrotic tissue was scored as follows: no fibrotic tissue = 0, superficial or focal fibrotic tissue = 1, superficial disseminated or deep local fibrotic tissue = 2, deep and widespread fibrotic tissue = 3.[17] All images taken from histological samples were obtained by means of a digital camera connected to a light microscope. The images were saved to a computer for original magnification. Histomorphometric analyses were performed using the Olympus DP71 software imaging system.

Statistical Analysis

Statistical analysis was performed with SPSS 23.0 for Windows software, IBM (Armonk, New York). Data for each group are expressed as mean ± standard deviation. Differences between the groups were detected using one-way ANOVA. Tukey's honestly significant difference test was used to determine the group that caused these differences, and $P < 0.05$ was considered statistically significant.

RESULTS

The NBF in the ZA-D-1 and ZA-D-2 groups, when compared with the control group, showed a statistically significant difference, but no statistically significant differences were detected between the treatment groups (Supplementary Digital Content, Table 1, http://links.lww.com/SCS/C679) (Figs. 2A–C and 3A–C).

When compared with the control group, the numbers of osteoblasts in the ZA-D-1 and ZA-D-2 groups were statistically significantly higher when compared with the controls. Moreover, no

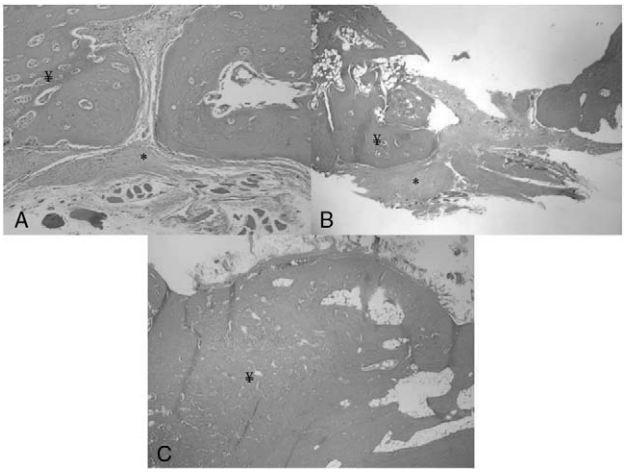

FIGURE 2. (A) Distracted area of the control (2×), (B) Zoledronic acid dosage 1 (2×) and (C) zoledronic acid dosage 2 (2×). ¥: New bone formation, *: Fibrosis.

statistically significant differences were detected between the ZA-D-1 and the ZA-D-2 groups for the osteoblast number, but numerically the osteoblast number detected was higher in the ZA-D-2 group (Supplementary Digital Content, Table 1, http://links.lww.com/SCS/C679) (Fig. 2A–C and 3 A–C). Fibrosis in the controls, when compared with the ZA-D-1 and ZA-D-2 groups, was found to be statistically significantly higher. In addition, the fibrosis values were found to be statistically significantly higher in the ZA-D-1 group than in the ZA-D-2 group (Supplementary Digital Content, Table 1, http://links.lww.com/SCS/C679) (Figs. 2A–C and 3A–C).

DISCUSSION

Distraction osteogenesis is an original method that was first described in 1905 but gained wide acceptance only after Dr Gavriil Ilizarov identified the physiologic and mechanical factors responsible for the successful regeneration of bone formation in the late 1980s. In dentistry, DO has been used for the restoration of congenital and/or acquired maxillofacial bone tissue defects and deficiencies. Despite its encouraging results, the long consolidation time for optimal new bone tissue formation can create complications, such as infection, pin loosening, pseudo-articulation/nonunion of the distracted fragments, refracture, late bowing, and a psychosocial burden on the patients.[11,12,17,18]

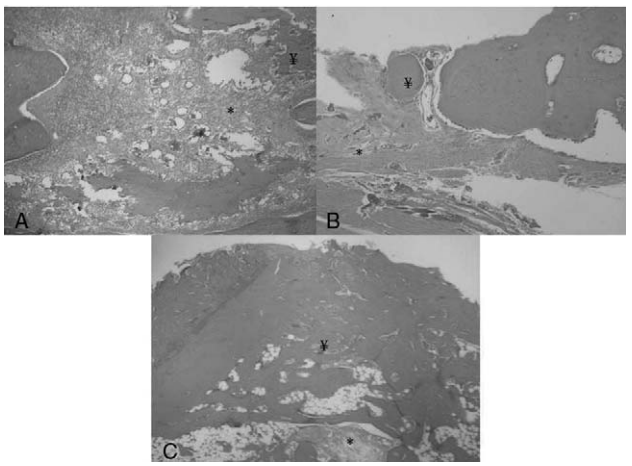

FIGURE 3. (A) Distracted area of the control (2×), (B) Zoledronic acid dosage 1 (2×), and (C) Zoledronic acid dosage 2 (2×). ¥: New bone formation, *: Fibrosis.

In order to decrease the DO period, research has been conducted to investigate the rate of bone distraction. A fast rate of bone distraction can reportedly reduce the quality of the newly regenerated bone, thereby negatively affecting the distracted area. However, at slow bone distraction rates, there is a risk of premature bone fragment integration, and the bone tissue lengthening may not be successful. Zoledronic acid is a strong BP for clinical use, and a single dose of zoledronic acid has been shown to have favorable effects in various models of bone tissue repair and healing.[13,18] Similarly in our study, zoledronic acid administration positively affected bone healing at both doses.

Distraction osteogenesis has been researched in many different animal models, including sheep, dogs, pigs, rats, and rabbits.[11,14,20–26] The rat DO method is a well-established experimental model, and has been used widely in DO studies, thanks especially to its ease of use and proper bone size.[27–32] In the light of the studies, we carried out our work on rats. In the study conducted by Leblein et al and Li et al, the number of subjects was evaluated as 8.[32,33] In the study conducted by Weng et al, the number of subjects was evaluated as 10.[34] Considering the possibility that some of the subjects may die during surgical procedures, in our study we determined the number of subjects as ten.

The aim of most previous research has been to induce new bone tissue formation, reduce the consolidation time, increase the bone quality and quantity, and decrease the risk of nonunion of the distracted fragments. For these reasons, researchers have studied the application of various methods or pharmacological agents to quicken the maturation of the new bone, with varying results. Different biomedical techniques, including pulsed electromagnetic fields, low-intensity pulsed ultrasound, and electrical stimulation, have also been evaluated by researchers. Moreover, growth factors, calcium sulfate, the transplantation of osteoblast-like cells, grafting with a demineralized bone matrix, and bone morphogenetic protein have also been studied.[12] In our study, according to the desire to search for a shortened consolidation period after DO, the possibility of using zoledronic acid is being investigated.

Bisphosphonates-related jawbone osteonecroses (ONJ) are defined as the remaining necrotic bone that occurs in patients who received BP for 8 weeks. Risk factors for this condition are known as tooth extractions, inflammation, and a number of drugs prescribed in oncological therapy, such as poor oral hygiene, steroids, antiangiogenics, chemotherapeutics. It is very difficult to treat ONJ and the patient's quality of life is affected. Although the incidence of ONJ varies among patients with cancer, most retrospective studies estimate that a minimum of 5% of IV BP users occur ONJ. At the Arkansas University Medical Center, 479 oncological patients were retrospectively analyzed and augen was observed in 25 patients who had received BP for an average of 4.4 years (range, 1–8 years). Ten of 25 patients used steroids in the month before diagnosis, 11 patients were reported to have received dental treatment before ONJ developed. In our study, the effect of bisphosphonate use in the treatment of DO was evaluated in patients using BPs, as jaw bone necroses occur frequently.[35,36]

Bisphosphonates are synthetic inorganic pyrophosphate analogs used for the treatment of various osteoclast-originating bone diseases, and work by organizing osteoclast apoptosis and restricting differentiation to mature osteoclasts, or by decreasing osteoclastic cell activity.[12,14] Bisphosphonates are involved in the mevalonate pathway through the obstruction of the farnesyl pyrophosphate synthase enzyme. The major pharmacodynamic effect of this drug is the inhibition of osteoclastogenetic bone tissue resorption, but it also has the potential for interacting with osteoblastic cells and promoting osteoblast proliferation and differentiation, causing

increased bone tissue formation.[12,14,19] In our study, the number of osteoblasts in the ZA-D-1 and ZA-D-2 groups was found to be statistically significantly higher when compared with controls. This situation shows us that BPs application increases osteoblastic activity.

Recently, the relationship between BPs and bone tissue has increasingly been the subject of investigation in the literature. Zoledronic acid is the most potent BP used clinically.[12,14,20] A single dose of zoledronic acid has been shown to have favorable effects in various models of bone tissue repair and healing.[13,18] Zoledronic acid also shows favorable effects on osteoblast maturation. For example, Omi et al investigated the effects of local BP administration in a rabbit tibial DO model, and showed that a local low-dose alendronate administration could be an effective method of improving bone formation.[21] In addition, Küçük et al[12] reported that the use of systemic and local alendronate may be effective in accelerating NBF in the distraction gap in rabbit mandibles. In another study, Tekin et al evaluated a postoperative local alendronate injection in the distraction gap in the first three days during a distraction procedure, and determined that it may be effective in the acceleration of NBF.[11] In a study by Pampu et al[37] showed that ZA had positive effects on the NBF, which may potentially shorten the consolidation period.

Similarly, in our study, it was observed that zoledronic acid applied groups had positive effects on bone healing. We think that this occurs because zoledronic acid increases osteoblast activity and decreases osteoclast activity.

CONCLUSIONS

In this experimental study, we aimed to evaluate the effects of a single dose of 0.1 mg/kg or 0.2 mg/kg systemically administered zoledronic acid on new bone tissue formation in a rat femoral DO model. A 0.375 mm/d, twice-daily, DO bone-lengthening protocol was performed according to the literature. The histomorphometric results showed 0.2 mg/kg single-dose zoledronic acid may be an effective method for generating new bone maturation in DO in rat femurs, when compared with the controls. Our histological results in the zoledronic acid treatment groups supported these results. Bisphosphonates are widely used in cancer treatment. Cancer disease is increasing day by day today. Due to this increase, the rate of osteonecrosis in bisphosphonates increases. Osteonecrosis is often seen in the jawbone. Therefore, a current issue was addressed in our study. The effects of bisphosphonates at different rates were examined histologically and no significant difference was obtained between 0.1 mg/kg and 0.2 mg/kg. More studies are needed for more precise information about bisphosphonates whose effects are examined at different rates.

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