Optimal increase in bone mass by continuous local infusion of alendronate during distraction osteogenesis in rabbits

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ABSTRACT

Several methods have been used to increase bone mass in distraction osteogenesis. Since bone resorption as well as regeneration is stimulated in the distracted segment, bisphosphonate can be a beneficial agent for distraction osteogenesis. Here, we examined the effects of bisphosphonate injected continuously into the regenerate on bone volume, and architectural and mechanical properties of distraction osteogenesis. The left tibia of Japanese White rabbits (n=66) was subjected to slow distraction using an external fixator. At the beginning of the consolidation phase, alendronate (7 μg/kg/day) was infused directly into the lengthened segment for 14 days using an osmotic pump. Control rabbits were infused with phosphate buffered saline (PBS). The tibiae were monitored weekly by soft X-ray and dual-energy X-ray absorptiometry (DXA). The animals were sacrificed at 4, 6, and 8 weeks after operation to examine bone mineral density (BMD) and cortical bone thickness (CBT) by peripheral quantitative computerized tomography (pQCT), while the mechanical property of the lengthened tibia was measured by three-point bending test. In PBS-infused control animals, bone mineral content around the lengthened segment began to decrease after the first week of consolidation phase, forming a tubular bone structure with thin cortex. Infusion of alendronate increased peak bone mineral content around the lengthened segment. At the end of the experiment, volumetric BMD, CBT and mechanical strength of the lengthened segment of the treatment group were approximately twice those of the control animals. Alendronate infused in this manner significantly prevented the osteopenia that critically began early in the consolidation phase, though the dose used in this study was relatively low and no adverse events were noted.

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Introduction

Distraction osteogenesis is used for limb lengthening and consists of osteotomy with subsequent slow progressive distraction [9,14,15,45]. The technique is used for the treatment of short bones with complex bone deformities, segmental bone loss and congenital or post-traumatic pseudoarthrosis [4,33]. To achieve significant lengthening, however, the patient must wear a bulky external fixator for a long period of time [33,44].

The process of distraction osteogenesis can be divided into three distinct phases; a lag phase, a distraction phase and a consolidation phase [18]. The lag phase is short and constant, while the distraction phase varies depending on the magnitude of lengthening. The consolidation phase is frequently longer than the distraction phase. For example, to achieve 10-cm lengthening, the lag phase is usually 7 days, distraction is 100–150 days, while the consolidation phase extends over 150 days. Thus, the overall duration of treatment is approximately 300 days [4,33,44].

There are a great number of studies in which growth factors were administered into the lengthened segment to shorten the treatment period by stimulating new bone formation [2,3,13,20,31]. However, some of them could not present significant effects. Moreover, it has been shown that bone resorption as well as regeneration was highly activated in the distracted segment [39]. It may be because new bone trabeculae are mechanically unloaded while wearing an external fixator [21,22]. Thus, bisphosphonates, which are potent anti-resorptive agents, must be a more advantageous candidate than growth factors in distraction osteogenesis. Little et al. [21–23] reported the effects of bisphosphonate on prevention of the fixator-related osteoporosis. In experimental studies, we also reported high dose of bisphosphonate was required to control highly-activated bone resorption during distraction osteogenesis [39]. Bisphosphonates have been applied to clinical distraction osteogenesis to rescue insufficiency of bone formation [17]. On the contrary, adverse events of bisphosphonate including osteonecrosis of the jaw [6,34,37] and osteopetrosis or atypical skeletal fragility [28,41,43] have recently emerged in the patients who had been treated with high dose of bisphosphonates. In particular, intravenous injection of bisphosphonate has been reported to cause renal toxicity.

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which can be sometimes life-threatening, as well as myalgia, fever and nausea [10]. Therefore, it would be ideal to establish a novel method of administration, which can minimize the dose of bisphosphonate in order to avoid those adverse events while maximizing the effect on the highly activated bone resorption during distraction osteogenesis.

Recently, we established a new method to deliver an agent continuously into the lengthened segment [1]. Using an osmotic pump in a rabbit model of distraction osteogenesis, fibroblast growth factor II (FGF-II) was continuously infused over two weeks at various phases. The results showed that FGF-II begun at the beginning of the consolidation phase had a significant effect to enhance bone formation. Using the same drug-delivery system, we examined the effects of minimum dose of alendronate on bone resorption and remodeling of the lengthened segment. Alendronate is a widely used bisphosphonate to treat osteoporosis all over the world [5,40]. Significant increases in bone mass and strength were achieved via the least dose of bisphosphonate that has ever been reported.

Materials and methods

Animals

Animal experiments were carried out on 66 male Japanese white rabbits, weighing 1.8 to 2.2 kg, purchased from Kitayama Labes Co. (Nagano, Japan). The experimental protocol was approved by the local animal protection agency and the ethics committee of the University of Tokushima.
The rabbits were anesthetized by intravenous injection of ketamine hydrochloride (20 mg/kg body weight) and xylazine (5 mg/kg body weight). After application of a unilateral external fixator (Orthofix M-100) to the left tibia using four self-taping screws, superiosteal osteotomy was performed between the second and third screw using a fine wire saw [1,18,39]. A small drill hole was made above the osteotomy and a 23-gauge needle was inserted obliquely into the marrow cavity until it reached the distal bone fragment. By slow distraction, the needle was pulled back and the tip was placed in the center of the lengthened segment (Fig. 1A). The needle was connected to a polyvinyl catheter, which was embedded in the subcutaneous tissue until use.

The lag phase was 7 days, followed by distraction at a rate of 0.35 mm every 12 h (0.7 mm/day). Distraction was continued for 14 days until lengthening of 10±0.1 mm was achieved. Following the completion of distraction, the animals were kept for 35 days until consolidation of the lengthened segment was obtained. At the beginning of the consolidation phase, the animals were anesthetized again for implantation of an osmotic pump (Alzet 2ML2, Durect Co. Cupertino, CA) in the subcutaneous tissue of the back, as described previously [1]. The pump was connected to the polyvinyl catheter that was embedded subcutaneously at the time of the primary operation, so that the solution in the pump was infused slowly into the center of the lengthened segment for 14 days, starting from the beginning of the consolidation phase (Fig. 2). Subsequently, the rabbits were divided into two experimental groups (33 animals each). Alendronate (7 μg/kg/day) was continuously infused in the one group and phosphate buffered saline (PBS) in the other.

Peripheral quantitative computerized tomography (pQCT)

Following sacrifice at 4, 6, and 8 weeks after the initial operation, the right and left tibiae were prepared for pQCT study. Using the Stratec pQCT (XCT-960A; Norland/Stratec, Fort Atkinson Pforzheim, USA/Germany), 18 slices of the lengthened left tibia was analyzed. Six slices within the lengthened segment and 6 slices above and below the osteotomy, each 1.67 mm in thickness, were analyzed. Six slices of the non-operated right tibia were used as controls.

Mechanical analysis

The mechanical properties of the lengthened tibiae were examined after the measurement of pQCT. The tibiae were cleaned of soft tissue and three-point bending strength was measured until failure with a support span of 40 mm between the sand third pin holes, using a servohydraulic material testing system (S-100; Shimazu, Kyoto, Japan) with a 1 kN load cell under displacement control (5 mm/min). The ultimate strength was determined as described previously [1,39].

Statistical analysis

Data were expressed as means±standard deviation (SD). Differences between groups were examined statistically by a Student’s t-test. P-values<0.05 were considered significant.

Results

Radiological findings

In all animals, lengthening of 10±0.1 mm was successfully achieved and bone consolidation was obtained by the end of the experiment. Radiologically, the lengthened segment in control animals showed a characteristic zone structure consisting of a
central radiolucent zone and two sclerotic zones at the beginning of consolidation (Fig. 1A), as described previously [18,39]. Fusion, shrinking and eventual resorption of the proximal and distal sclerotic zones were noted during the consolidation phase (Figs. 1B–E). In addition, periosteal and endosteal bone remodeling proceeded to form a tubular bone with thin cortices in all control animals (Fig. 1E), which just looked as osteopenia of the lengthened segment.

Continuous infusion of alendronate did not disturb bone union of the lengthened segment, but rather strongly inhibited the bone resorption seen in PBS-infused animals during the consolidation phase (Figs. 1F–I). Inhibition of bone resorption by alendronate resulted in delay of periosteal and endosteal bone remodeling. The diameter of the lengthened segment was maintained and alendronate strongly inhibited stress-shielding osteopenia, especially in the distal half of the lengthened segment (Figs. 1F–I). There were no radiological abnormalities on the metaphysis such as osteopetrosis and growth arrest line (Fig. 3B).

DXA studies

Fig. 3 shows the serial changes in BMC of the lengthened segment and adjacent original tibia above and below osteotomy (each 10 mm in length). In the beginning of consolidation phase, the BMC of the lengthened segment was increased. Subsequently, the BMC in all three regions decreased gradually and progressively until the end of the experiment, consistent with the radiological findings. Continuous local infusion of alendronate into the lengthened segment significantly increased BMC in all three regions compared to PBS-infused animals at 5 weeks after operation (Fig. 4). Although BMC gradually decreased later than this time point, the BMC of the lengthened segment was still seven times higher in alendronate-infused animals than in PBS-infused animals at the end of the experiment ($P<0.01$). There was no difference in BMC at the corresponding region of the contralateral tibia between PBS- and alendronate-infused animals. The patterns of changes in areal BMD measured by DXA were similar to those of BMC in either the operated or contralateral tibia (data not shown).

pQCT studies

Fig. 5 shows cross-sections of the lengthened tibia imaged by pQCT at 4, 6 and 8 weeks after the initial operation over the consolidation phase. By 4 weeks, the original tibiae below and above osteotomy was surrounded by periosteal new bone, exhibiting double layer cortices in both the PBS- and alendronate-infused rabbits (Fig. 5A). In contrast, the lengthened segment was composed of a homogeneously fine cancellous bone with no apparent cortex at this stage in both groups, while small cavities of bone marrow were noted in the center of the lengthened segment. The lengthened segment has already had higher bone density in alendronate-treated animals at this stage. By 6 weeks after operation, the bone marrow cavity in the lengthened segment expanded gradually to form a thin cortex in PBS-infused animals (Fig. 5B). Continuous infusion of alendronate strongly inhibited this endosteal bone resorption. Consequently, the cortical bone thickness of the lengthened segment of alendronate-infused animals was approximately twice that of the control PBS-infused animals at 8 weeks after operation (Fig. 5C. See also Fig. 7A). Within the lengthened segment, there was a trend that the central slices showed higher BMD compared to the peripheral slices at 6 and 8 weeks. This is likely due to the radiodense mineralized zone noticeable in PBS-infused animals.

Fig. 6 shows volumetric BMD calculated by pQCT analysis. Consistent with the DXA studies, local administration of alendronate into the lengthened segment significantly prevented loss of BMD in all three areas. The volumetric BMD of the lengthened segment in alendronate-infused animals was $112\%$ at 4 weeks ($P=0.35$), $189\%$ at 6 weeks ($P<0.01$) and $152\%$ at 8 weeks ($P<0.01$) after operation, relative to the corresponding values of PBS-infused animals. There were no differences in BMD at the corresponding region of the contralateral tibia between PBS- and alendronate-infused animals at any time points (data not shown).
Mechanical analysis

As shown in Fig. 7B, the ultimate strength of the lengthened tibia of alendronate-infused animals was approximately twice stronger than that of the PBS-infused control animals at 6 and 8 weeks after operation. There were no significant differences in ultimate strength of the contralateral tibiae between the two groups at any time points (data not shown).

Discussion

We have found that bone mineral content around the lengthened segment still increased during the first one week of consolidation phase followed by the slow but definite decrease over the rest of the phase (Figs. 4B–D, solid lines). This was obvious even on radiographs (Figs. 1B–E). It looks as if bone mass reaches its peak in the course of distraction osteogenesis. This observation may correspond with the fact that bone morphogenetic protein (BMP)-2 and BMP-4 which were over-expressed throughout the distraction phase and attenuated immediately after the completion of distraction in a rat femoral lengthening model [35]. Infusion of alendronate continuously into the distraction segment at the beginning of the consolidation phase significantly increased this peak bone mineral content through the accelerated inclination of bone mineral content and the extended phase of bone mineral apposition (Fig. 3, dashed lines). pQCT examination demonstrated that the increase in bone mineral content was given by the promoted formation and/or preservation of thick cortical structure of the distracted segment, which eventually led to a significant increase in mechanical property.

In distraction osteogenesis, induced bone is basically woven bone, is not exposed to the mechanical loading under the presence of an external fixator and is destined to be rapidly resorbed by osteoclasts without being remodeled into lamellar bone. It has been demonstrated that promoted bone formation consequently led to bone resorption in accordance with the cross-talk between osteoblast and osteoclast lineages [16,25]. Recently Okamoto et al. reported that abundant BMPs might stimulate not only osteoblasts but osteoclasts during bone development [30]. These results suggest that bone resorption is also highly activated in distraction osteogenesis. Indeed, a characteristic zone structure in the lengthened segment results from both activated bone formation and simultaneously activated resorption of new bone regenerates [18,39]. In the clinical practice, stress-shielding osteopenia is frequently observed in the lengthened bone, especially in the distal bone segment below osteotomy. Eyres et al. [11] demonstrated in pediatric limb lengthening that the mean BMD in the distal bone segment was decreased by 44.2% in the tibia and by 61.0% in the femur. Maffuli et al. [24] reported that the BMC values of bone adjacent to the distraction site were decreased by 40% in 6 of 11 patients. Furthermore, local osteopenia after limb lengthening persisted for 2 years after surgery, although the external fixator had already been removed.

In order to prevent local osteopenia during limb lengthening, Little et al. [21–23] tested the effects of various types of bisphosphonates in rabbit experiments. They demonstrated that a single dose of pamidronate (1 mg/kg) at operation increased BMD in the lengthened segment by 8%, proximal bone segment above osteotomy by 11%, and distal bone segment below osteotomy by 14% [22]. In another study, they reported that intravenous administration of a higher dose of pamidronate (3 mg/kg) was more effective in increasing the areal

Fig. 5. pQCT images around the lengthened segment of PBS-infused animal (top row) and alendronate-infused animal (bottom row) at (A) 4 weeks, (B) 6 weeks and (C) 8 weeks after operation. Coloring of cross-section represents bone mineral density (BMD) as indicated by a standard scale on the left.

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BMD of the lengthened segment (43%), the proximal bone segment (40%), and distal bone segment (39%) [21]. We recently demonstrated that weekly-administered high-dose (0.4 mg/kg/w) minodronate, one of the most potent bisphosphonates, increased radiological bone density of the lengthened segment by ~230% and the strength by ~330% through inhibition of the over-resorption in new bone regenerates in a rabbit model of distraction osteogenesis [39]. Although no momentarily adverse events were noted in the animal experiment, systemic administration of high-dose bisphosphonate is not recommended in the clinical application.

To minimize the dose of bisphosphonate but at the same time obtain the equal or more effects, we administered comparatively low-dose alendronate (7 μg/kg/24 h; total dosage ~ 200 μg/14 days) directly into the lengthened segment in the present study. Although this dose was rather high compared to the clinical dosage of alendronate (oral 35 or 70 mg/w), we considered it necessary to inhibit the highly activated bone resorption during distraction osteogenesis. The increases in mean areal BMD at the end of experiment in the lengthened segment, that in the proximal segment above osteotomy and that in the distal segment below osteotomy were 83%, 37% and 43%, respectively, whilst the increases in volumetric BMD were 94%, 48%, and 52%, respectively. These effects on bone mass were more prominent despite the lower dose of bisphosphonate compared to any other previous reports. In contrast, neither decreased tibial growth, metaphyseal abnormality (Fig. 3) nor changes in the BMC of contralateral tibia (Fig. 4E) was evident throughout the present study. One of the metaphyseal abnormalities under an exposure of bisphosphonate is under-modeling with osteopetrosis (Fig. 3C) [12,42]. The other is growth arrest line which is observed both clinically and experimentally as radiodense line on the metaphysis [12,42]. Omi et al. reported that weekly local injection of 7.5-μg/kg alendronate into the lengthened segment still caused growth arrest lines in 6 out of 7 operated tibiae in rabbit model [32]. Despite the presence of adverse event, this dose increased only 32.8% in BMC and 22.9% in BMD of the lengthened segment with no statistical differences. Intermittent bolus administration of bisphosphonates should result in leakage of the agents outside the lengthened bony segment, though bisphosphonates have a high affinity for bone matrix to be absorbed. Since bone matrix is gradually formed in the lengthened segment during distraction osteogenesis, bisphosphonate slowly infused can be incorporated continuously into the newly-synthesized bone matrix to persist and can act as an inhibitor of bone resorption for long period of time. Alendronate has been shown to have higher affinity compared to the other bisphosphonates [19,27] and to be more efficiently uptaken into osteoclasts [7]. Although we did not measure the blood concentration of alendronate as a result of leakage in the present study, continuous administration of alendronate will certainly minimize the leakage, which avoids the risk of adverse events. We have recently shown infusion of Indian ink from the beginning of the consolidation phase, using the same osmotic pump, resulted in its accumulation within the lengthened segment, whereas the same dye infused during the distraction phase or lag phase mostly leaked outside the lengthened segment [1]. The timing of the administration that we have used in the present study would be ideal, if we consider those previous results and the facts that bone mineral content began to decrease 1 week after the beginning of consolidation phase.

Although the local osteopenia during distraction osteogenesis was clearly diminished without any systemic effects in this delivery system, long-term impact of bisphosphonate on local bone remodeling would remain to be considered. McDonald et al. [26] reported, in a rat fracture model, that prolonged bisphosphonate dosing affected the remodeling, which eventually led to inferior bone material properties. The remodeling process, which is osteoclast dependent, is necessary for converting woven bone into mature lamellar bone that is mechanically superior [8]. Our results showed bone parameters began to decrease after the administration of alendronate ceased, indicating that osteoclasts already function at this stage. It is still
unknown, however, whether this process also remodels woven bone and/or cartilaginous tissues formed early in the regenerate, and whether this delivery system also minimizes the impairment of osteoblastic activity. Future studies are needed to clarify the long-term effects of alendronate on the remodeling process.

In conclusion, continuous local infusion of alendronate into the lengthened segment during the beginning of consolidation phase improved morphological and mechanical properties of the lengthened segment. The timing of administration and the applied dosage were experimentally confirmed to optimize effects of alendronate. We consider that this method can be applicable to clinical treatment by using an alternative delivery method and could contribute to enhancing bone mass and shortening treatment time of limb lengthening by distraction osteogenesis. Further studies are required before clinical application to prove the efficacies in the case of long lengthening, and to justify the dose and concentration of bisphosphonates and position of the catheter.

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References


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