

Effect of Dosing Interval Duration of Intermittent Ibandronate Treatment on the Healing Process of Femoral Osteotomy in a Rat Fracture Model

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Abstract The effects of bisphosphonate treatment schedule on fracture healing have not previously been tested. We evaluated the effect of ibandronate dosing interval duration on healing following surgical “fracture” (osteotomy) using a rat femoral fracture model. Six-week-old rats ($n = 160$) underwent osteotomy and were then allocated into vehicle control (CNT) or an ibandronate treatment group: 5 $\mu\text{g}/\text{kg}$ daily (DAY, 5 days/week), 75 $\mu\text{g}/\text{kg}$ once every 3 weeks (I-3), 150 $\mu\text{g}/\text{kg}$ once every 6 weeks (I-6), resulting in the same total ibandronate dose over the study. Rats were killed after 6 or 18 weeks. At 18 weeks, all fracture lines had disappeared in the CNT and I-6 groups; approximately 10% of fracture lines remained in the DAY and I-3 groups. Ibandronate-treated groups showed large callus areas around the fractures, which shrank between 6 and 18 weeks after surgery; the extent of shrinkage decreased with shorter dosing interval. In histomorphometry, callus remodeling was suppressed by ibandronate; this became more apparent at shorter dose intervals. The structural properties of osteotomized femora were increased in the DAY group compared with CNT, but intrinsic material properties reduced inversely and became closer to those of CNT in response to increased dosing interval. Ibandronate induced formation of large calluses around osteotomies but delayed woven bone

remodeling into lamellar bone and reduced intrinsic material properties in a rat fracture model. Extending the dosing interval of intermittent ibandronate treatment appeared to reduce the suppression of callus remodeling caused by ibandronate, which would have delayed healing after osteotomy.

Keywords Fracture healing · Bisphosphonate · Intermittent treatment · Dosing interval · Callus remodeling

Bisphosphonates are strong inhibitors of bone resorption and are widely used as effective therapeutic agents for a variety of bone diseases with high bone resorption, such as metastatic bone disease, Paget disease, and osteoporosis [1–5]. Currently, they are the most popular therapeutic agents for osteoporosis because they consistently decrease the incidence of osteoporotic fragility fractures [6–10]. Nitrogen (N)-containing bisphosphonates may persist on the bone surface for considerable periods of time, which has led to the widespread use of intermittent dosing in clinical practice. For the oral N-containing bisphosphonates risedronate and alendronate, this has resulted in a move from daily to weekly administration of a dose equivalent to seven times the standard daily dose [11–14].

Ibandronate is an N-containing bisphosphonate that has been developed specifically for administration with long dose-free intervals [15] and has been approved in the United States and Europe as a once-monthly oral regimen. The antifracture efficacy of ibandronate has been demonstrated with both daily dosing and intermittent treatment, with a dose-free interval of greater than 2 months [16]. In addition, once-monthly ibandronate has shown therapeutic equivalence to daily ibandronate for bone mineral density (BMD) gains at the lumbar spine and the hip with good safety and tolerability [17].

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As fractures may occur in patients undergoing treatment for osteoporosis, clinicians must be aware of the effects of therapeutic agents for osteoporosis on the fracture healing process. We previously reported the effects of antiresorptive agents such as bisphosphonates [18–21], eel calcitonin (elcatonin) [22], and selective estrogen receptor modulator (SERM) [21] on fracture healing. These agents delay the fracture healing process in response to the extent of callus remodeling suppression, although restoration of the mechanical strength of fractured bone is not impaired.

Intermittent bisphosphonate dosing regimens are more common for osteoporosis treatment than daily dosing [13]; however, the effects of the dosing interval on the fracture healing process have never been tested. We conducted the current study to evaluate the effects of the dosing interval of ibandronate on the healing process, especially callus remodeling, geometrical changes, and mechanical properties of fractured bone, using a rat femoral fracture model that imitates fracture development through osteotomy.

Materials and Methods

Materials

Female Sprague-Dawley rats ($n = 160$) aged 6 weeks were purchased from Japan SLC (Hamamatsu, Japan) and acclimated for 3 weeks to local vivarium conditions ($24 \pm 2^\circ\text{C}$ and 12-h light–dark cycle). During the experimental period animals were housed in cages (988 cm^2 in floor area and 18 cm in height) and allowed free access to water and a pelleted commercial rodent diet (Oriental Yeast, Tokyo, Japan). The experimental protocol was approved by the Kagawa University Animal Study Committee.

A powder form of [1-hydroxy-3-(methylpentylamino)-propylidene] bisphosphonic acid sodium salt (ibandronate; F. Hoffmann-La Roche, Basel, Switzerland) was dissolved in isotonic saline and the pH adjusted to 7.4. The solution was stored in a normal refrigerator (4°C). The doses are expressed as micrograms per kilogram of free acid equivalents of ibandronate. For all animals, the volume administered was 1 mL/kg, injected subcutaneously.

Experimental Design

Animals were randomly allocated into four groups based on body weight: control (CNT, saline vehicle), DAY (daily ibandronate 5 $\mu\text{g}/\text{kg}$, 5 days/week), I-3 (ibandronate 75 $\mu\text{g}/\text{kg}$ every 3 weeks), and I-6 (ibandronate 150 $\mu\text{g}/\text{kg}$ every 6 weeks). At the initiation of treatment, femoral osteotomy and fixation were performed in the same manner as previously reported [18–21, 23, 24]. Briefly, a transverse osteotomy was made at the midshaft of the left femur, the

fragments were contacted and stabilized, then the intramedullary was fixed using a stainless steel wire (diameter 1.5 mm). The wire was cut on the surface of the intercondylar groove to avoid restriction of motion of the knee joint. “Osteotomy,” therefore, describes the surgical procedure and “fracture” denotes the break in the bone resulting from osteotomy. Unrestricted activity was allowed after recovery from anesthesia. Body weights were measured weekly, and treatment dosages were adjusted accordingly. Dosing was initiated immediately after osteotomy and continued at 5 days per week until death; saline vehicle was given daily except for scheduled dosing of ibandronate to animals in the I-3 and I-6 groups. Total doses of ibandronate were equivalent in the three treatment groups. In order to analyze bone formation, double labeling was performed for all surviving animals by subcutaneous administration of calcein (6 mg/kg; Wako, Osaka, Japan) 7 and 2 days prior to necropsy. Rats were killed by exsanguination from the abdominal artery under general anesthesia at 6 or 18 weeks after the surgery, at the end of the intermittent dosing interval and before the next scheduled dose.

Evaluations

X-ray Photography

Right femora were excised and dissected free of soft tissues, and intramedullary wires were extracted. Anteroposteriorly, soft X-ray radiographs of the femora were taken (20 kV, 100 μA , 60 s; $\mu\text{FX-1000}$; Fujifilm, Tokyo, Japan). The presence of fracture lines was assessed by three orthopedic surgeons and considered “healed” if agreed on by at least two of the surgeons.

All femora were randomly divided into two evaluation groups. The first group was undecalcified, assigned for peripheral quantitative and computed tomography (pQCT), mechanical testing, contact micrograph, and histomorphometrical measurements. The other group was decalcified and assigned for tartrate-resistant acid phosphatase (TRAP) staining.

pQCT Analysis

Specimens were frozen at -80°C and wrapped in gauze soaked in isotonic saline until pQCT measurement. After thawing at room temperature, the right femora were scanned by pQCT (Norland/Stratec XCT Research SA; Stratec Medizintechnik, Pforzheim, Germany). The bones were placed horizontally inside a glass tube and scanned using a voxel size 0.12 mm. The scan line was adjusted using the scout view of the pQCT system. For analysis, a constant threshold of $464 \text{ mg}/\text{cm}^3$ was used to separate the bone area from the marrow. The volumetric total BMD (mg/cm^3) of

the fracture plane was calculated. The cross-sectional moment of inertia (CSMI, mm^4) was also calculated; however, the threshold value (464 mg/cm^3) was applied to enhance the accuracy of the CSMI calculated through pQCT in this fracture model.

Mechanical Testing

After pQCT scanning, the femora were tested for mechanical properties by a three-point bending method using a materials testing machine (MZ500S; Maruto, Tokyo, Japan). A femur was placed on two lower support bars (15 mm apart) with the anterior surface facing upward. The fracture plane was centered at the loading point, and load was applied at a rate of 2.5 mm/min until breakage. From the load versus displacement curve, the structural mechanical properties of the osteotomized bone were determined as ultimate load (maximum force that the specimen sustained), stiffness (the slope of the linear portion of the load vs. deformation curve before failure), and work to failure (the area under the load vs. deformation curve before failure). As previously described, because these structural parameters depend on the size and geometry of the testing specimens, they were normalized using CSMI to obtain intrinsic material properties such as ultimate stress (MPa), elastic modulus (MPa), and toughness (MJ/m^3), which are independent of cross-sectional size and shape [25].

Histologic Preparation and Contact Microradiograph

After mechanical testing, the proximal segments of the fracture were repositioned, fixed in 70% ethanol, stained in Villanueva bone stain, dehydrated in increasing concentrations of ethanol, defatted in xylene, and embedded in methyl methacrylate. Two 200- μm -thick cross sections were cut using a diamond microtome saw (SP1600; Leica, Nussloch, Germany) in an area within 500 μm from the original fracture line and then ground to 100 μm thickness for digital contact microradiographs (100 kV, 2 mA, 5 min; $\mu\text{FX-1000}$; Fujifilm) and 30 μm thickness for histomorphometry.

The remaining distal segments of the fracture were fixed in 10% cold neutral buffered formalin for 3 days and then decalcified in 10% ethylenediaminetetraacetic acid (EDTA) at 4°C for 4 weeks. Specimens were embedded in paraffin, and 5- μm -thick cross sections were cut in an area within 500 μm from the original fracture line. The section was stained with TRAP using a leukocyte acid phosphatase kit (Sigma, St. Louis, MO).

Histomorphometry

Histomorphometric analyses were performed with a semiautomated digitizing image analyzer. The system comprised a light or epifluorescent microscope and a digitizing pad

coupled with computer and histomorphometric software (System Supply, Nagano, Japan). Polarized light was applied to distinguish lamellar bone from woven bone. Total area (T.Ar, mm^2) and callus area (Cl.Ar, mm^2) were measured at $\times 10$ magnification, and percent callus area ($\% \text{Cl.Ar} = \text{Cl.Ar} / \text{T.Ar} * 100, \%$) was calculated. Further measurements were made at $\times 100$ magnification in four standardized quarters in the anterior, posterior, medial, and lateral aspects as previously described [18]. Callus surface (Cl.S), single-labeled surface (sLS), double-labeled surface (dLS), and interlabeling width (Ir.L.Wi) were measured at the callus. Diffuse labeled area was excluded from the measurement. Mineral apposition rate (MAR, $\mu\text{m/day}$), mineralizing surface (MS/Cl.S, $\%$), bone formation rate (BFR/Cl.S, $\%$), and percent lamellar area (Lamellar/Cl.Ar) were calculated. Osteoclast measurements were also performed at $\times 100$ magnification in the four standardized quarters. The number of osteoclasts (N.Oc) was determined and N.Oc/Cl.S (#/mm) calculated.

Statistical Analysis

Statistical computation of data was performed using the statistical package Stat View 5.0 (SAS Institute, Cary, NC). Differences among treatment groups were tested by one-way analysis of variance (ANOVA). If a significant difference was obtained, differences between the means of the two groups were tested by Fisher's protected least significant difference. $P < 0.05$ was considered significant.

Results

No differences were found in body weight among all groups during the study period. The animals resumed normal activity within a few days after surgery. From 160 animals at the beginning of the study, 11 were excluded because of technical failure, postsurgical infection, or death. Finally, 74 and 75 animals were evaluated and analyzed at 6 and 18 weeks after surgery, respectively.

Radiologic Findings

Soft X-ray images (Fig. 1) showed that although fracture lines had disappeared in almost 90% of the CNT group, they were still present in more than half of the ibandronate-treated animals at 6 weeks postosteotomy. At 18 weeks, all fracture lines had disappeared in the CNT and I-6 groups; however, approximately 10% of fracture lines remained in the DAY and I-3 groups (Table 1).

Contact microradiographs (Fig. 2) at 6 and 18 weeks after osteotomy revealed that the ibandronate treatment groups had visually larger calluses than CNT; however, at 18 weeks the callus sizes of all groups appeared to be

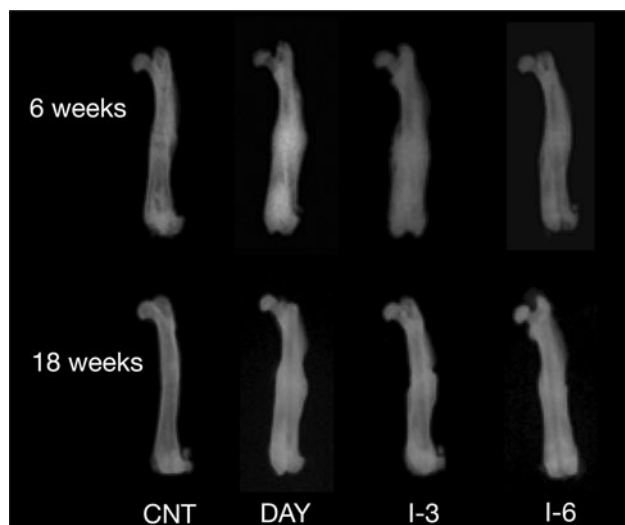


Fig. 1 Soft X-ray photography of fractured femora. Fracture lines had disappeared in almost 90% of the control group but were still present in more than half of ibandronate-treated groups at 6 weeks postfracture. At 18 weeks all fracture lines had disappeared in control and I-6 groups; however, about 10% of fracture lines remained in the DAY and I-3 groups

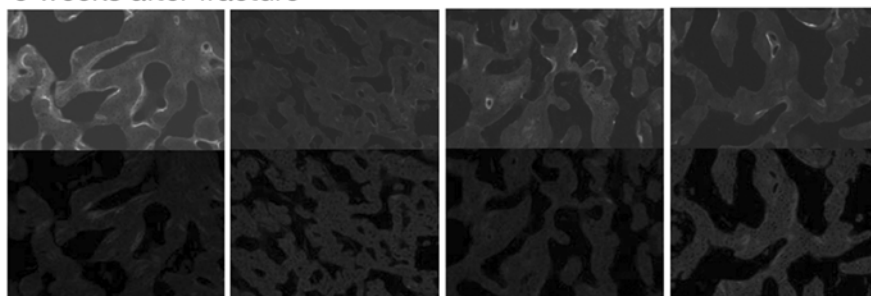
Table 1 Visible fracture lines in control (CNT), DAY, I-3, and I-6 groups

^a Data in parentheses are number of animals with visible fracture line/total number of animals in treatment group

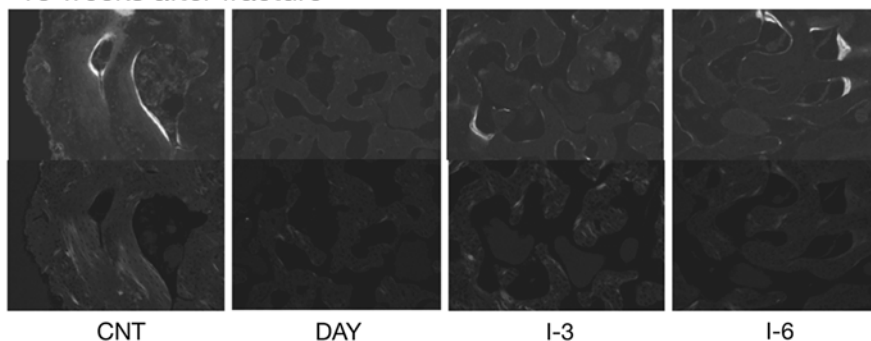
| Visible fracture lines (%) ^a | CNT | DAY | I-3 | I-6 |
|---|----------------------------|-----------------------------|-----------------------------|-----------------------------|
| 6 weeks after osteotomy | 12.5 (<i>n</i> = 2/16) | 57.9 (<i>n</i> = 11/19) | 78.9 (<i>n</i> = 15/19) | 50.0 (<i>n</i> = 10/20) |
| 18 weeks after osteotomy | 0 (<i>n</i> = 0/20) | 17.6 (<i>n</i> = 3/17) | 11.1 (<i>n</i> = 2/18) | 0 (<i>n</i> = 0/20) |

Fig. 2 Contact microradiographs of cross-sectional specimens at the fracture plane. At 6 and 18 weeks after osteotomy ibandronate treatment groups had visually larger calluses than CNT. At 18 weeks the callus sizes of all groups appeared to be smaller than at 6 weeks and new cortical shell was apparent, which was still porous in the DAY group

A 6 weeks after fracture



B 18 weeks after fracture



smaller than at 6 weeks and new cortical shell was apparent, which was still porous in the DAY group.

pQCT Analysis

Bone mineral content (BMC) around the fracture site was significantly increased with ibandronate treatment vs. CNT ($P < 0.05$), and the increase was dependent on a shortened ibandronate administration interval at both 6 and 18 weeks after osteotomy (Table 2). However, no significant differences were found between groups in volumetric BMD at 6 or 18 weeks. CSMI, a geometrical parameter of cross-sectional shape, was also significantly increased by ibandronate versus CNT ($P < 0.05$) (Table 2); however, these CSMI increases tended toward a numerical reduction with increasing treatment interval.

Mechanical Testing

At 6 weeks after osteotomy, stiffness was significantly higher in DAY than in CNT or I-3 femora ($P < 0.05$).

Table 2 pQCT analysis

| | CNT (n = 16) | DAY (n = 19) | I-3 (n = 18) | I-6 (n = 20) |
|---------------------------|-----------------|-------------------------------------|--------------------------------|---------------------------|
| 6 weeks after osteotomy | | | | |
| BMC (mg) | 12.89 ± 0.89 | 24.43 ± 1.70 ^{C, I-6} | 21.85 ± 1.31 ^{C, I-6} | 16.67 ± 0.80 ^C |
| BMD (mg/cm ³) | 973.52 ± 15.12 | 1,012.59 ± 19.83 | 981.77 ± 14.48 | 1,001.19 ± 23.70 |
| CSMI (mm ⁴) | 24.13 ± 3.78 | 63.36 ± 9.49 ^{C, I-6} | 55.62 ± 7.08 ^{C, I-6} | 35.04 ± 3.55 |
| 18 weeks after osteotomy | (n = 20) | (n = 17) | (n = 18) | (n = 20) |
| BMC (mg) | 12.50 ± 0.76 | 27.35 ± 1.94 ^{C, I-3, I-6} | 21.33 ± 1.35 ^C | 20.47 ± 1.10 ^C |
| BMD (mg/cm ³) | 1,217 ± 12.17 | 1,204.16 ± 13.12 | 1,211.33 ± 9.06 | 1,224.72 ± 13.32 |
| CSMI (mm ⁴) | 14.25 ± 1.51 | 44.92 ± 6.22 ^{C, I-3, I-6} | 29.10 ± 3.60 ^C | 26.82 ± 2.46 ^C |

Data are mean values ± standard error, with significant differences from CNT, DAY, I-3, and I-6 indicated by C, D, I-3, and I-6, respectively ($P < 0.05$, Fisher's projected least significant difference)

Ultimate load and work to failure were numerically, but not significantly, higher in DAY than in CNT femora; no significant differences between the ibandronate dosing groups were seen. When these data were normalized using the CSMI, to exclude the effect of increased callus volume due to the treatment, the intrinsic material properties, such as ultimate stress and elastic modulus, were significantly decreased by ibandronate treatment ($P < 0.05$ vs. CNT). Ultimate stress was significantly reduced in I-3 versus I-6 femora ($P < 0.05$), but no other significant differences were noted between the dosing groups. Intrinsic material properties

became closer to those of the CNT group in response to an increased interval of ibandronate administration (Table 3).

Among the structural mechanical properties at 18 weeks after osteotomy, only ultimate load in DAY femora was significantly higher than in CNT ($P < 0.05$). Intrinsic material properties were significantly decreased by ibandronate treatment ($P < 0.05$ vs. CNT); the only significant difference between ibandronate dosing groups was in elastic modulus in DAY vs. I-6 femora ($P < 0.05$). Increasing the interval of ibandronate administration lessened the decreases in ultimate stress and elastic modulus.

Table 3 Biomechanical properties of fractured femur

| | CNT (n = 16) | DAY (n = 19) | I-3 (n = 18) | I-6 (n = 20) |
|----------------------------------|------------------|----------------------------------|------------------------------|-----------------------------|
| 6 weeks after osteotomy | | | | |
| Structural mechanical properties | | | | |
| Ultimate load (N) | 83.7 ± 8.4 | 129.1 ± 27.9 | 75.6 ± 22.8 | 91.04 ± 8.76 |
| Stiffness (N/mm) | 7,919 ± 368 | 10,446 ± 937 ^{C, I-3} | 8,028 ± 936 | 8,302 ± 707 |
| Work to failure (N-mm) | 74,113 ± 16,199 | 104,456 ± 28,619 | 65,334 ± 14,525 | 74,587 ± 11,407 |
| Intrinsic material properties | | | | |
| Ultimate stress (MPa) | 36.5 ± 2.9 | 24.5 ± 4.6 ^C | 15.1 ± 3.8 ^{C, I-6} | 30.9 ± 4.0 |
| Elastic modulus (MPa) | 27,913 ± 3,925 | 12,732 ± 2,102 ^C | 10,750 ± 1,217 ^C | 17,386 ± 1,717 ^C |
| Toughness (MJ/m ³) | 4,255 ± 756 | 3,204 ± 674 | 2,391 ± 660 | 4,287 ± 946 |
| 18 weeks after osteotomy | (n = 20) | (n = 17) | (n = 18) | (n = 20) |
| Structural mechanical properties | | | | |
| Ultimate load (N) | 140.3 ± 10.9 | 224.0 ± 27.4 ^C | 184.8 ± 18.6 | 171.9 ± 10.3 |
| Stiffness (N/mm) | 12,134 ± 786 | 13,347 ± 788 | 12,038 ± 737 | 13,219 ± 818 |
| Work to failure (N-mm) | 120,539 ± 18,359 | 153,667 ± 29,530 | 123,590 ± 24,171 | 106,071 ± 14,529 |
| Intrinsic material properties | | | | |
| Ultimate stress (MPa) | 79.7 ± 4.0 | 53.3 ± 3.9 ^C | 60.9 ± 4.6 ^C | 60.0 ± 4.5 ^C |
| Elastic modulus (MPa) | 62,593 ± 3,825 | 26,343 ± 3,547 ^{C, I-6} | 31,989 ± 2,690 ^C | 37,541 ± 4,707 ^C |
| Toughness (MJ/m ³) | 7,581 ± 1,001 | 4,868 ± 666 ^C | 5,260 ± 1,019 | 4,730 ± 714 ^C |

Data are mean values ± standard error, with significant differences from CNT, DAY, I-3, and I-6 indicated by C, D, I-3, and I-6, respectively ($P < 0.05$, Fisher's projected least significant difference)

Histomorphometry

After osteotomy, observations in the CNT group at the 6- and 18-week time points indicated that T.Ar and T.Cl.Ar decreased as a result of shrinkage of the fracture callus and bone formation parameters decreased. In addition, Lamellar/Cl.Ar was 63% at 6 weeks in the CNT group, reaching 100% at 18 weeks after osteotomy in this group (Table 4; Fig. 3).

Following ibandronate treatment, bigger callus formation and delayed callus remodeling were observed in an apparent interval-dependent manner. T.Ar and T.Cl.Ar were significantly higher in DAY than in CNT femora at 6 weeks postosteotomy ($P < 0.05$), with nonsignificant numerical decreases in T.Ar and T.Cl.Ar from DAY toward CNT levels in the I-3 and I-6 groups (Table 4). DAY femora showed significantly larger T.Ar than all other treatment groups at 18 weeks after osteotomy ($P < 0.05$). At 18 weeks, T.Ar and T.Cl.Ar were numerically reduced in all groups compared with the 6-week time point. T.Ar and T.Cl.Ar for I-3 and I-6 were between DAY and CNT values, although all groups were significantly different from CNT. These data suggest less shrinkage of the fracture callus, especially in the DAY group compared with the CNT group.

Lamellar/Cl.Ar was significantly reduced in ibandronate-treated groups compared with CNT, and the extent of the reduction lessened as the ibandronate administration interval was shortened (Table 4). Similarly, bone remodeling

parameters, such as the fluorochrome label-based bone formation parameter or the static bone resorption parameter, were significantly decreased by ibandronate treatment vs. CNT ($P < 0.05$); and these decreases became more apparent in response to shortening of the administration interval. Interval-dependent numerical changes were observed for MAR, BFR/Cl.S, MS/Cl.S, and N.Oc/Cl.S, which are all

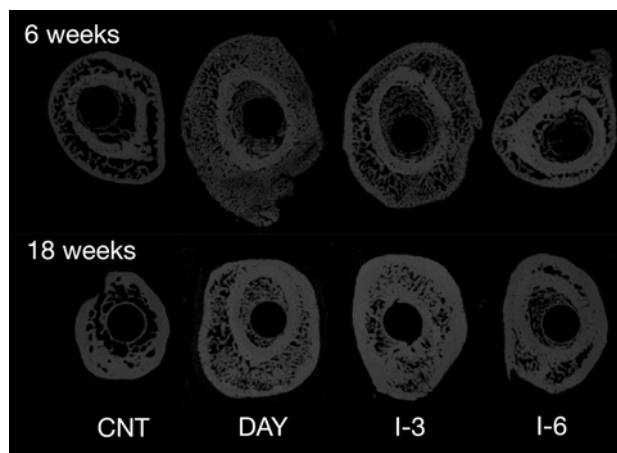


Fig. 3 Photomicrographs of the callus at 6 and 18 weeks after osteotomy, under epifluorescence and polarized light. At 6 weeks The labeled surface and lamellar bone in the callus were less in all treatment groups than CNT. At 18 weeks lamellar bone formation was active in the CNT and I-6 groups, while less lamellar and more woven bone were observed in the DAY group than in any other groups

Table 4 Bone histomorphometry in fracture callus

| | CNT (n = 16) | DAY (n = 19) | I-3 (n = 18) | I-6 (n = 20) |
|---|-----------------|-------------------------------------|-------------------------------|----------------------------|
| 6 weeks after osteotomy | | | | |
| T.Ar (mm ²) | 22.78 ± 1.72 | 29.67 ± 2.31 ^C | 29.89 ± 1.90 ^C | 26.40 ± 2.21 |
| T.Cl. Ar (mm ²) | 15.63 ± 1.38 | 21.39 ± 2.26 ^C | 19.62 ± 2.19 | 18.75 ± 1.54 |
| Lamellar/callus (%) | 62.6 ± 7.2 | 31.3 ± 8.8 ^{C, I-3, I-6} | 43.0 ± 11.1 ^{C, I-6} | 52.9 ± 4.5 ^C |
| MAR (µm/day) | 3.17 ± 0.28 | 0.39 ± 0.26 ^{C, I-3, I-6} | 2.15 ± 0.45 ^C | 2.38 ± 0.12 |
| BFR/Cl.S (mm ³ /mm ² /year) | 0.302 ± 0.040 | 0.002 ± 0.001 ^{C, I-6} | 0.050 ± 0.018 ^C | 0.093 ± 0.011 ^C |
| MS/Cl.S (%) | 25.63 ± 2.04 | 1.12 ± 0.22 ^{C, I-6} | 5.23 ± 1.81 ^C | 9.58 ± 1.67 ^C |
| N.Oc/Cl.S (#/mm) | 4.26 ± 1.10 | 0.75 ± 0.31 ^C | 0.71 ± 0.33 ^C | 1.38 ± 0.34 ^C |
| 18 weeks after osteotomy | (n = 20) | (n = 17) | (n = 18) | (n = 20) |
| T.Ar (mm ²) | 15.62 ± 0.85 | 24.59 ± 1.76 ^{C, I-3, I-6} | 20.62 ± 1.12 ^C | 20.01 ± 0.87 ^C |
| T.Cl.Ar (mm ²) | 9.69 ± 0.77 | 16.63 ± 1.74 ^C | 13.89 ± 1.21 ^C | 13.33 ± 0.60 ^C |
| Lamellar/callus (%) | 100 | 67.8 ± 15.2 ^{C, I-3, I-6} | 86.2 ± 11.0 ^{C, I-6} | 95.9 ± 4.1 |
| MAR (µm/day) | 2.81 ± 0.25 | 0.25 ± 0.18 ^{C, I-3, I-6} | 0.94 ± 0.49 ^C | 2.32 ± 0.39 |
| BFR/Cl.S (mm ³ /mm ² /year) | 0.17 ± 0.034 | 0.008 ± 0.007 ^{C, I-6} | 0.010 ± 0.005 ^C | 0.048 ± 0.010 ^C |
| MS/Cl.S (%) | 15.19 ± 3.13 | 0.86 ± 0.16 ^{C, I-6} | 2.17 ± 0.46 ^C | 5.71 ± 1.11 ^C |
| N.Oc/Cl.S (#/mm) | 4.10 ± 1.07 | 0.40 ± 0.07 ^{C, I-6} | 0.94 ± 0.10 ^C | 2.10 ± 0.32 ^C |

Data are mean values ± standard error, with significant differences from CNT, DAY, I-3, and I-6 indicated by C, D, I-3, and I-6, respectively ($P < 0.05$, Fisher's projected least significant difference)

sensitive parameters of histomorphometry, although these changes were not significant between the ibandronate treatment groups (Table 4).

Discussion

Dosing convenience is a key element in the effective management of any chronic disease and is particularly important in the long-term management of osteoporosis. Less frequent dosing with any medication may enhance compliance, thereby maximizing the effectiveness of therapy. Bisphosphonate therapy effectively reduces the risk of osteoporotic fractures [26–28]; however, fracture protection depends critically on adherence and persistence. Bisphosphonate treatments with extended dosing intervals are more convenient for patients than daily dosing; patients' satisfaction with treatment is improved, as is their likelihood of remaining on therapy, ultimately improving the clinical benefits of treatment [29, 30]. These extended dosing intervals for bisphosphonates are based on the “total dose concept” that bone protection by bisphosphonates is a function of the total effective dose of the compound regardless of the regimen used, whether continuous or cyclical intermittent [10]. However, the total dose concept may not be applicable universally but depends on the properties of each individual drug. For example, weekly risedronate and alendronate are administered at the same cumulative dose as daily administration (equivalent to seven times the standard daily dose) [11–14], whereas monthly ibandronate (150 mg) is twice the cumulative daily dose. Notably, however, a lower dose of monthly ibandronate (100 mg), which is closer to the daily cumulative dose, provided the same benefits as daily dosing in the MOBILE study [17]. While this concept has been proven in both osteoporotic animal models and human osteoporosis, the effects of the dosing interval duration for bisphosphonates on the fracture healing process have never been tested.

The histomorphometric findings of the present study showed inhibition of both bone resorption and formation and less lamellar bone in the callus area in ibandronate-treated groups, indicating delayed callus remodeling into lamellar bone. Larger callus volume and cross-sectional area (moment of inertia) in ibandronate-treated groups appeared to be a compensation mechanism for the delay in remodeling woven bone into lamellar bone, which is structurally and mechanically superior to woven bone. These observations are consistent with our previous studies using other bisphosphonates, incadronate and alendronate [18–21].

The primary aim of this study was to examine the effect of extended dosing intervals for ibandronate on the healing

process following osteotomy in the femoral fracture model in rats. Our results demonstrated that extension of the dosing interval reduced suppression of callus remodeling of woven bone into lamellar bone, suggesting that extended bisphosphonate dosing intervals may bring the healing process for “fractures” following osteotomy closer to what is natural. The total dose concept may not therefore be applicable for this healing process in rats. There are several explanations for this phenomenon. Firstly, although bisphosphonates bind to bone surfaces because of a high affinity to hydroxyapatite, the amount of deposited bisphosphonate may be limited in a single dosing regimen. Thus, the total amount of compound within the bone may be less in the intermittent treatment groups than in the daily treatment group. Secondly, bisphosphonates have different bone binding affinities, which, in addition to resorption rate, most likely determine the rate of release from bone. Ibandronate has a lower binding affinity than zoledronate and alendronate but a greater bone binding affinity than risedronate [31, 32]. Thus, in the intermittent treatment groups, a substantial amount of deposited ibandronate may have been desorbed from the bone during the dosing interval, resulting in less suppression of callus remodeling in the intermittent ibandronate treatment groups vs. the DAY group. Additionally, the greater the dosing interval, the longer the recovery time for bone remodeling, as evidenced by the histomorphometric data for the DAY vs. the intermittent ibandronate treatment groups. These findings are supported by our previous fracture study using incadronate, in which bisphosphonate concentrations in bone were measured in the fracture callus in comparison with contralateral intact bone. We found a time-dependent decrease in incadronate concentration in the fracture callus after its withdrawal, whereas it remained unchanged in contralateral intact bone, indicating that the release of incadronate from bone depends on the rate of local bone turnover [18–20].

The current study could not establish whether the different intermittent ibandronate dosing schedules, administered to the same total cumulative dose, showed comparable antifracture efficacy. Some caution should be exercised when interpreting the clinical relevance of these results in terms of treatments for osteoporosis. Young rats were used (8–9 weeks old at the time of treatment initiation), which is consistent with previous studies in the rat fracture model [18–21, 23, 24, 33]; however, in terms of clinical relevance, this is a weakness of the study as the fracture healing process in these animals will differ from that of patients with osteoporosis. In addition, the total dose of ibandronate used in the DAY group was higher than the clinical daily dose for osteoporosis treatment; however, the doses used were chosen to inhibit the high metabolic turnover in this rat model and to produce sufficient callus to detect differences

in mechanical parameters between the daily and intermittent groups. In addition, how the treatment intervals in this rat study compare with those used in patients with osteoporosis is unclear. If the histomorphometric remodeling period is considered, a dosing interval of 3–6 weeks in the rat is equivalent to approximately 3–6 months in humans, assuming that the remodeling period in the rat is about 30 days. Alternatively, based on the life span of the animal (approximately 2 years for rats), 3–6 weeks in rats is equivalent to 2–4 years in humans, which would suggest that the dosing intervals used in this study are longer than those in the treatment of osteoporosis in humans.

Conclusion

Based on histomorphometric and biomechanical evaluations of osteotomized femora in rats treated with the same total doses of ibandronate in three different dosing intervals (daily, every 3 weeks, and every 6 weeks), we conclude the following: (1) ibandronate treatment induced formation of large fracture calluses but delayed woven bone remodeling into lamellar bone and reduced intrinsic material properties, (2) shortening the dosing interval of ibandronate treatment without changing the total dose enhanced suppression of callus remodeling and appeared to delay the healing process after osteotomy, and (3) extending the dosing interval of intermittent ibandronate treatment appeared to reduce the suppression of callus remodeling caused by ibandronate that would have delayed healing after osteotomy.

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