Pamidronate in Children with Osteogenesis Imperfecta: Histomorphometric Effects of Long-Term Therapy

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Context: Intravenous pamidronate treatment is beneficial to children and adolescents with osteogenesis imperfecta (OI), but the effects of prolonged therapy are not well characterized.

Objective: The objective of this study was to assess the effect of long-term pamidronate treatment on the bone tissue of children and adolescents with OI.

Design: This is an observational study on OI patients receiving iv pamidronate for more than 4 yr.

Setting: The study was carried out in a pediatric metabolic bone research unit.

Patients: Patients were 25 moderately to severely affected OI patients (seven girls) aged 1.4–15.3 yr at baseline.

Intervention: Intervention was cyclical iv pamidronate at a dose of 9 mg/kg/yr.

Subjects and Methods

Osteogenesis Imperfecta (OI) is a genetic disorder with increased bone fragility and low bone mass. The most commonly used classification distinguishes four clinical types (1). OI type I comprises patients with absence of bone deformities. Type II is lethal in the perinatal period. OI type III is the most severe form in children surviving the neonatal time. Patients with mild to moderate bone deformities and variable short stature are classified as OI type IV. In the majority of patients with OI, the disease can be linked to mutations in one of the two genes coding for collagen type I α-chains (COL1A1 and COL1A2) (1). Recently, three disease entities (named OI types V, VI, and VII) have been identified that have a similar phenotype as the other types of OI but are not associated with collagen type I mutations (1).

The bisphosphonate pamidronate has a beneficial effect in children and adolescents with severe OI (1). It has been reported that this treatment increases lumbar spine areal bone mineral density (BMD) and metacarpal cortical width, decreases fracture rates, and improves mobility, even though a recent small placebo-controlled trial on low-dose pamidronate was unable to reproduce some of these results (2). On the level of the bone tissue, pamidronate increases cortical width and cancellous bone volume and suppresses bone turnover (3).

The available studies on pamidronate therapy in OI dealt with the initial benefits and mostly reported results for the first 2 yr of treatment or even shorter periods of time. The effects of prolonged treatment on the bone tissue of pediatric OI patients are unknown at present. This lack of information makes it difficult to establish a treatment regimen that maximizes the benefits and limits the potential for side effects. Therefore, in the present study, we analyzed the bone tissue effects when pamidronate therapy was continued up to an average treatment period of 5.5 yr.

Main Outcome Measures: Iliac bone biopsy and lumbar spine bone mineral density measures were obtained at treatment start, after 2.7 ± 0.5 yr (mean ± sd), and after 5.5 ± 0.7 yr of therapy.

Results: Average areal bone mineral density increased by 72% in the first half of the observation period, but by only 24% in the second half. Mean cortical width and cancellous bone volume increased by 87 and 38%, respectively, between baseline and the first time point during treatment (P < 0.001 for all changes). Thereafter, cortical width did not change significantly, but there was a trend (P = 0.06) toward higher cancellous bone volume. Average bone formation rate on trabecular surfaces decreased by 70% after pamidronate treatment was initiated and showed a trend (P = 0.08) toward a further decline in the second part of the study interval.

Conclusion: The gains that can be achieved with pamidronate treatment appear to be largely realized in the first 2–4 yr. (J Clin Endocrinol Metab 91: 511–516, 2006)

Abbreviations: BMD, Bone mineral density; NTX, cross-linked N-telopeptides of type I collagen; OI, osteogenesis imperfecta.

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Biopsies were not performed in patients with a body weight less than 10 kg or who presented an elevated risk for anesthesia.

The OI type IV group did not include patients who fulfilled the Silence criteria for OI type IV, but who could be further classified as having OI type V, VI, or VII on the basis of our expanded classification (1). Patients were included in the present evaluation if iliac bone samples of sufficient quality were available from the start of therapy, after 2–4 yr of treatment, and after more than 4 yr of pamidronate therapy.

At the time of the present analysis, measurable bone biopsy specimens at all three time points had been obtained from 25 patients (seven girls, 18 boys). The diagnostic distribution was as follows: OI type I, n = 10; OI type III, n = 2; OI type IV, n = 13. The low number of patients with OI type III is explained by the difficulty of obtaining bone biopsies in these patients, who often present with an elevated anesthetic risk. Age at the start of pamidronate treatment ranged from 1.4–15.3 yr (mean ± sd, 8.0 ± 3.8 yr). At the time of the second and third biopsies, these patients had received pamidronate therapy for 2.7 ± 0.5 yr (range 2.0–4.0 yr) and 5.5 ± 0.7 yr (range 4.3–6.5 yr), respectively. Collagen type I mutations were found in 21 of these patients. In four patients, no collagen type I mutation was detectable by full sequence analysis of the COL1A1 and COL1A2 genes, but a diagnosis of OI was made on the basis of typical clinical findings (dentinogenesis imperfecta, blue sclerae). During the time interval from 6 months before the first biopsy to the last biopsy of this study, 17 patients had at least one (range one to nine) fracture of a lower extremity long-bone (tibia or femur).

Histomorphometric results in the study group were compared with those of two control groups that have been described earlier (3). The first control group comprised 58 subjects between 1.5–22.9 yr of age, whose results have been published before (6). These individuals had undergone iliac bone biopsies during minor orthopedic procedures. The second control group consisted of 123 children and adolescents (59 girls, 64 boys; age 1.4–21 yr, median 8.4 yr) with a diagnosis of OI type I (n = 47), OI type III (n = 22), or OI type IV (n = 54). The data from this second control group were used to derive age- and OI type-specific comparative data, as explained elsewhere. Histomorphometric data from 70 of these patients have been published earlier (7). The study was approved by the Shriners Hospital Institutional Review Board, and informed consent was obtained from legal guardians.

Treatment. Pamidronate was administered iv on 3 consecutive days in all patients. As described in detail elsewhere, the timing and dosage of these procedures (6). Wall thickness was not measured in the present study, because reversal lines are difficult to visualize in severe OI. Consequently, activation frequency could not be determined. Measurements were carried out using a digitizing table with Osteomeasure software (Osteometrics, Inc., Atlanta, GA). Some parameters were not measurable in a few samples due to inadequate sample preservation. Therefore, the number of patients in whom a complete data set was available differs between parameters. Nomenclature and abbreviations follow the recommendations of the American Society for Bone and Mineral Research (9).

Bone densitometry

Bone densitometry was performed in the antero-posterior direction at the lumbar spine (L1–L4) using a Hologic QDR 2000W or 4500A device (Hologic, Inc., Waltham, MA). The traditional densitometric parameter, areal BMD, is a composite measure of three-dimensional mineral density and bone length in the antero-posterior direction (10). A size-independent measure of three-dimensional density was derived by calculating the ratio between bone mineral content and the extrapolated external volume of the measured bones (volumetric BMD). This was done as described by Carter et al. (10) using the formula: volumetric BMD = (bone mineral content)/(projection area) (11).

Anthropometric and biochemical measurements

Height and weight measurements were converted to age- and sex-specific z-scores on the basis of reference data published by the Centers for Disease Control and Prevention (11). Urinary cross-linked N-telopeptides of type I collagen (NTX) were quantified by enzyme-linked immunosorbent assay (Osteomark; Ostex, Seattle, WA) using the second void sample of the morning. Results for urinary NTX to creatinine ratios in OI patients were expressed as a percentage of age-specific mean values using published reference data (12). Patients were fasting at the time of urine sampling.

Statistical analyses

Variables were tested for normal distribution using the Kolmogorov-Smirnov test. Normally distributed data were expressed as mean ± sd. Geometric means and geometric sd were calculated for nonnormally distributed variables. These variables were log transformed before performing tests that require normal distribution. The difference between results at baseline and at the two time points during therapy was tested for significance using ANOVA for repeated measures. Post hoc comparisons were performed using Bonferroni’s adjustment.

### TABLE 1. Anthropometric and densitometric results at baseline (biopsy 1), after 2–4 yr of pamidronate treatment (biopsy 2), and after more than 4 yr of pamidronate treatment (biopsy 3)

<table>
<thead>
<tr>
<th>Duration of PAM (yr)</th>
<th>At time of biopsy 1</th>
<th>At time of biopsy 2</th>
<th>At time of biopsy 3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.7 (0.5)</td>
<td>5.5 (0.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (z-score)</td>
<td>-3.1 (2.2)</td>
<td>-3.1 (2.3)</td>
<td>-2.2 (2.4)</td>
<td>0.71</td>
</tr>
<tr>
<td>Weight (z-score)</td>
<td>-1.7±(1.5)</td>
<td>-1.1±(1.5)</td>
<td>-1.1±(1.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Densitometry (lumbar spine)</td>
<td>7.5k±h (1.9)</td>
<td>17.9k±h (1.6)</td>
<td>27.0k±c (1.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bone mineral content (g/cm²)</td>
<td>26.6±h (7)</td>
<td>35.5±c (9)</td>
<td>41.5±c (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Areal BMD (g/cm²)</td>
<td>0.32±c (0.12)</td>
<td>0.55±c (0.13)</td>
<td>0.68±c (0.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Volumetric BMD (mg/cm³)</td>
<td>64.3±h (17)</td>
<td>89.3±c (17)</td>
<td>106±c (16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>NTX/creatinine (% of age- and sex-specific reference mean)</td>
<td>113±k (59)</td>
<td>52±c (20)</td>
<td>44±c (18)</td>
</tr>
</tbody>
</table>

Values are mean (sd), n = 25 for all measures. P values represent the significance of the difference of results at the three time points (ANOVA).

a Significant difference to result at 2–4 yr of PAM.
b Significant difference to result at more than 4 yr of PAM.
c Significant difference to pretreatment result.
d For BMC geometric mean (geometric sd) are given.
To compare histomorphometric measures in OI patients to those of healthy subjects, results of each patient were expressed as a percentage of the published age-specific mean value (6). To compare the results of the study group to those from OI patients who had not received bisphosphonate therapy, the age-specific mean value in bisphosphonate-naive patients was first derived by linear regression of each histomorphometric parameter with age. This analysis was performed for the three OI types separately. For parameters that did not significantly vary with age in a given OI type, the mean value of the entire group was used. The results of each study patient were then expressed as a percentage of the age-specific mean value in untreated patients.

All tests were two-tailed, and throughout the study \( P < 0.05 \) was considered significant. These calculations were performed using the SPSS software, version 11.5, for Windows (SPSS, Inc., Chicago, IL).

**Results**

The participants of the present study were, on average, very short (Table 1). The height z-score did not change significantly during the observation period, whereas the weight z-score increased. Average lumbar spine bone mineral content more than tripled during the study period, whereas the projection area enlarged by only 58% (Table 1). This translated into total increases in areal and volumetric BMD of 113 and 66%, respectively. Both areal and volumetric BMD increased more from baseline until the time of biopsy 2 (72 and 45%, respectively) than in the second half of the observation period (24 and 14%, respectively). The urinary NTX to creatinine ratio, expressed as a percentage of the result expected for age and sex in a healthy population, decreased by 61% between baseline and the first time point during treatment, but did not change significantly thereafter.

External bone size (core width) increased significantly during pamidronate therapy (Fig. 1 and Table 2). However, the increase was similar to the changes expected with growth. Consequently, no significant change in core width was observed when expressed as a percentage of the age- and OI-type-specific averages or of the healthy age-specific mean value (Fig. 2). Mean cortical width increased by 87% between the baseline sample and sample 2. Cortical width did not change significantly thereafter.

Average cancellous bone volume increased by 38% between baseline and sample 2 (Table 2). There was a trend toward a further increase between samples 2 and 3 (\( P = 0.06 \)). This was due entirely to an increase in trabecular number, whereas no significant changes occurred in trabecular thickness. At baseline, calcified cartilage was found in three of the 25 biopsy specimens. In these three samples, cartilage volume (relative to bone volume) ranged from 0.2–6.9%. At baseline, calcified cartilage was found in three of the 25 biopsy specimens. In these three samples, cartilage volume (relative to bone volume) ranged from 0.2–6.9%. At the following time points, the number of samples containing cartilage rose to five and 12, respectively (\( P < 0.01 \) for changes from sample one to three, Wilcoxon test).

All bone surface-related measures of bone formation (osteoid surface, osteoblast surface, mineralizing surface, bone formation rate) decreased between baseline and sample 2 (Table 2 and Fig. 2). Differences between samples 2 and 3 were not significant, but there was a trend toward a further decrease in bone formation rate (\( P = 0.08 \)). Bone formation rate in sample 3 corresponded to 24% of the age-specific average value for subjects without bone disorder and to 13% of the result expected for OI patients not receiving medical treatment (Fig. 2). During therapy, the fraction of osteoid seam length showing mineralizing activity was significantly smaller than at baseline (Table 2). Therefore, mineralization lag time was prolonged during pamidronate treatment, even though mineral apposition rate and osteoid thickness did not change significantly.

During pamidronate therapy, osteoclast surface decreased by 46%, whereas eroded surface did not change significantly (Table 2). The change in osteoclast surface between samples 2 and 3 was not significant.

To assess the influence of disease severity on the effects of long-term pamidronate treatment, patients with OI type I (\( n = 10 \)) were compared with the more severely affected patients with OI types III and IV (\( n = 15 \)). Changes during treatment were calculated as the differences between results at the time of biopsy 1 and 3. Patients with OI type I grew better. Their gain in height z-score was 0.35 ± 0.48 (mean ± sd), compared with a loss of 0.43 ± 0.90 in OI types III and IV (\( P = 0.02 \)). OI type I patients had a larger increase in biopsy sample size (core width: +4.2 ± 2.3 mm vs. +0.7 ± 1.9 mm; \( P = 0.002 \)), cortical width (+635 ± 402 \( \mu \)m vs. +349 ± 259 \( \mu \)m; \( P = 0.04 \)), trabecular bone volume per tissue volume
TABLE 2. Results for histomorphometric parameters at baseline (sample 1), after 2 to 4 yr (sample 2), and after more than 4 yr (sample 3) of pamidronate treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Core width (mm)</td>
<td>17</td>
<td>3.1 (1.1)</td>
<td>4.1 (2.0)</td>
<td>4.9 (2.3)</td>
</tr>
<tr>
<td>Cortical width (μm)</td>
<td>25</td>
<td>391 (1.6)</td>
<td>733 (1.5)</td>
<td>808 (1.6)</td>
</tr>
<tr>
<td>Bone volume per tissue volume (%)</td>
<td>20</td>
<td>8.4 (1.5)</td>
<td>11.6 (1.8)</td>
<td>15.6 (1.6)</td>
</tr>
<tr>
<td>Trabecular thickness (μm)</td>
<td>25</td>
<td>101 (21)</td>
<td>107 (32)</td>
<td>106 (31)</td>
</tr>
<tr>
<td>Trabecular number (mm)</td>
<td>20</td>
<td>0.83 (1.5)</td>
<td>1.12 (1.5)</td>
<td>1.58 (1.4)</td>
</tr>
<tr>
<td><strong>Formation parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoid thickness (μm)</td>
<td>25</td>
<td>4.9 (1.3)</td>
<td>4.1 (0.7)</td>
<td>4.3 (1.1)</td>
</tr>
<tr>
<td>Osteoid surface per bone surface (%)</td>
<td>25</td>
<td>51 (14)</td>
<td>29 (11)</td>
<td>21 (10)</td>
</tr>
<tr>
<td>Osteoblast surface per bone surface (%)</td>
<td>24</td>
<td>20 (1.8)</td>
<td>4.6 (2.4)</td>
<td>4.7 (3.2)</td>
</tr>
<tr>
<td>Mineralizing surface per bone surface (%)</td>
<td>15</td>
<td>25 (1.5)</td>
<td>7.5 (1.9)</td>
<td>4.1 (3.4)</td>
</tr>
<tr>
<td>Mineralization lag time (d)</td>
<td>15</td>
<td>14 (1.6)</td>
<td>22 (1.6)</td>
<td>32 (2.2)</td>
</tr>
<tr>
<td>Adjusted apposition rate (μm/d)</td>
<td>15</td>
<td>0.36 (0.14)</td>
<td>0.21 (0.11)</td>
<td>0.18 (0.12)</td>
</tr>
<tr>
<td>Bone formation rate per bone surface (μm²-μm²⁻¹-yr⁻¹)</td>
<td>15</td>
<td>56 (1.7)</td>
<td>17 (2.0)</td>
<td>9 (3.9)</td>
</tr>
<tr>
<td><strong>Resorption parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoclast surface per bone surface (%)</td>
<td>24</td>
<td>1.03 (2.3)</td>
<td>0.75 (2.1)</td>
<td>0.56 (2.4)</td>
</tr>
<tr>
<td>Eroded surface per bone surface (%)</td>
<td>25</td>
<td>19 (9)</td>
<td>16 (9)</td>
<td>17 (9)</td>
</tr>
</tbody>
</table>

Values are mean (SD). P values represent the significance of the difference of results at the three time points (ANOVA).

* Significant difference to result at more than 4 yr of pamidronate.

+ Significant difference to pretreatment result.

- Geometric mean (geometric SD) are given.

* Significant difference to result at 2–4 yr of pamidronate.

(+16.6 ± 7.9% vs. +4.3 ± 5.6%; P < 0.001), and trabecular number (+1.1 ± 0.4 /mm vs. 0.6 ± 0.5 /mm; P = 0.04). The two groups were not significantly different with regard to changes in the other parameters shown in Tables 1 and 2.

**Discussion**

In this study we longitudinally evaluated iliac bone samples from young OI patients who had undergone bone biopsy before pamidronate therapy, after 2 to 4 yr of treatment and after more than 4 yr of treatment. Large initial treatment effects were observed, as reported before (3). However, only minor differences were detected between samples 2 and 3, corresponding to an average pamidronate treatment duration of 2.7 and 5.5 yr, respectively.

It should be noted that these histomorphometric results do not necessarily reflect a direct effect of pamidronate on the bone tissue. The treatment also has been reported to increase muscle force, decrease fracture rates, and increase mobility (1). All of these changes are likely to have an influence on histomorphometric results. On the other hand, the majority of patients sustained at least one major lower extremity fracture shortly before or during the observation period. Episodes of immobilization before biopsy might lead to transient bone loss, thus increasing the variability of histomorphometric findings.

The main structural effects of pamidronate are to increase cortical width and cancellous bone volume. The time course in these parameters resembles that of lumbar spine BMD insofar as changes were more marked in the first half of the observation period than in the second. Thus, cortical width was not significantly different after an average of 5.5 yr than what it was after 2.7 yr, suggesting that a new steady-state had been achieved. Cancellous bone volume showed a trend toward further increase, which was entirely explained by a higher number of trabeculae. This probably reflects the effect of pamidronate on endochondral bone growth. Endochondral growth is normally characterized by the production of a large number of thin primary trabeculae, most of which are rapidly resorbed during the conversion of primary into secondary spongiosa (13). Pamidronate therapy increases the number of trabeculae, presumably because fewer primary trabeculae are resorbed (14).

Pamidronate therapy inevitably reduces cancellous bone turnover. The present study suggests that this effect may depend on the duration of treatment, as bone formation rate showed a trend toward a further decline in the second treatment period. This histomorphometric finding contradicted somewhat the urinary NTX/creatinine results, which did not show a significant change after the initial treatment period. However, biochemical markers of bone turnover are influenced by the rate of longitudinal growth and cortical modeling activity, and therefore are probably less sensitive to changes in cancellous bone remodeling than dynamic histomorphometry.

The decreasing remodeling activity may explain why more samples contained calcified cartilage during pamidronate treatment. Growth plate cartilage probably is not removed completely during the conversion of primary to secondary spongiosa. It has been suggested that this residual growth plate cartilage might cause bone fragility, based on the case report of a school-age boy who had received severalfold higher pamidronate doses than the patients described here (15). At present, we have no indication that the increased amount of calcified cartilage caused clinical problems in our patients, but this possibility must be monitored.

Similar to our previous histomorphometric studies in older OI patients, none of the participants of the present study had signs of a mineralization defect (3). Mineralization lag time was prolonged during therapy, but there was no accumulation of osteoid. Therefore, the prolonged mineral...
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Fig. 2. Results for histomorphometric parameters at baseline (sample 1), after 2–4 yr (sample 2), and after more than 4 yr (sample 3) of pamidronate treatment. Results are expressed as a percentage of the average results in subjects without bone disorders, and as a percentage of the mean result in OI patients matched for age and OI type who had not received pamidronate before biopsy. The error bars represent SE values. a, Significant difference to pretreatment result; b, Significant difference to result at 2–4 yr of pamidronate. Abbreviations: C.Wi, Core width; Ct.Wi, cortical width; BV/TV, bone volume per tissue volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; O.Th, osteoid thickness; OS/BS, osteoid surface per bone surface; Ocs/BS, osteoclast surface per bone surface; ES/BS, eroded surface per bone surface; MS/BS, mineralizing surface per bone surface; MS/OS, mineralizing surface per osteoid surface; MAR, mineral apposition rate; Mlt, mineralization lag time; Aj.AR, adjusted apposition rate; BFR/BS, bone formation rate per bone surface.

ization lag time is a sign of sluggish remodeling activity rather than of a mineralization defect.

Patients with a less severe phenotype (OI type I) had a better histomorphometric response to pamidronate treatment than more severely affected patients (OI types III and IV), as evidenced by larger gains in bone size, cortical width, and trabecular bone volume, even though the effect on bone metabolism was similar between these patient groups. As OI type I patients also grew better, this observation is in accordance with the concept that the pamidronate effect is dependent on growth. Bone size and cortical width are determined by modeling on periosteal and endocortical surfaces, whereas trabecular number is influenced by endochondral ossification. All of these processes are more active in more rapidly growing individuals. Therefore, growth will amplify any drug effect on these metabolic processes.

In conclusion, the present study shows that pamidronate treatment has a marked effect on bone tissue during the first 2–4 yr of treatment, but that only small changes occur thereafter. The gains that can be achieved with this treatment approach thus appear to be largely realized in the first 2–4 yr. This raises the question whether it makes sense to continue treatment beyond that period. The observations that pamidronate treatment is associated with calcified cartilage accumulation and delayed bone healing after osteotomies may be seen as arguments for limiting the time of treatment (16). Nevertheless, there is a lack of data on whether treatment discontinuation is clinically more beneficial than prolonging the treatment period beyond 4 or 5 yr. These are key issues concerning the use of pamidronate in this patient group that need to be addressed in future studies.

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