Long-bone changes after pamidronate discontinuation in children and adolescents with osteogenesis imperfecta

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Abstract

Cyclical intravenous pamidronate is a widely used symptomatic therapy in moderate to severe osteogenesis imperfecta (OI). The effects of treatment discontinuation on long bone development have not been characterized. In this observational study we used peripheral quantitative computed tomography to assess the radius at the distal metaphysis and at the diaphysis in 23 young OI patients (11 female) who had received pamidronate for at least 3 years. Measurements were performed twice, at the time of treatment discontinuation (when the age of the patients ranged from 5.9 to 21.3 years) and at an average of 1.9 years (range 1.5 to 2.4 years) later. At the time of pamidronate discontinuation, all but one of the patients who were below 15 years of age (n=14) had a positive age- and sex-specific z-score for bone mineral content (BMC) at the metaphysis, resulting in a mean z-score of +2.0 (SD=1.0) for this subgroup. In contrast, patients aged 15 years or older (n=9) had an average metaphyseal BMC z-score of −1.5 (SD=1.5). After pamidronate discontinuation, metaphyseal BMC z-score decreased by an average of 2.4 (SD=2.0) in the whole group. The change in BMC z-score was growth-dependent, as BMC z-scores decreased by about 2 or more in all patients in whom distal radius growth plates were open when pamidronate was discontinued. In contrast, none of the 11 patients with closed distal radius growth plates experienced a decrease in metaphyseal BMC z-score by more than 2. At the diaphysis, the average BMC z-score was low at the time of the last pamidronate infusion [z-score −1.7 (SD=1.4)]. After pamidronate discontinuation, the average diaphyseal BMC z-score decreased by only 0.3 (SD=0.4). In summary, this study shows that the effect of pamidronate discontinuation is much larger at the radial metaphysis than at the diaphysis and is dependent on growth. Metaphyseal bone tissue added by longitudinal growth after treatment discontinuation has a lower density than tissue created during treatment. It is possible that this produces zones of localized bone fragility after pamidronate treatment is stopped in growing children.

Keywords: Bisphosphonates; Children; Metabolic bone disease; Osteoporosis; Peripheral quantitative computed tomography

Introduction

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 encoding collagen type I alpha 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].

Cyclical intravenous treatment with 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 bone mineral density (BMD) and metacarpal cortical width, decreases fracture rates and improves mobility [1–3]. Pamidronate is now used worldwide to treat children and adolescents with moderate to severe forms of OI.

Nevertheless, it is unclear for how long pamidronate treatment should be continued. One might argue that, as OI is a lifelong disorder, the symptomatic treatment with pamidronate should never be stopped. On the other hand, there is lingering concern about the long-term consequences of the treatment. Bisphosphonates are buried in the skeleton where they have a
half-life of many years [4]. Any adverse effects that might arise from the presence of the drug in the bones may therefore manifest late and persist for a long time.

Given the fact that the long-term consequences of the treatment during the growing years are unknown, it may be desirable to limit the exposure of young OI patients to pamidronate. In a previous study, we therefore evaluated the effects of treatment discontinuation in young OI patients who had received pamidronate treatment for several years [5]. This showed that the gains in lumbar spine bone mass that had been achieved during pamidronate therapy were maintained for at least 2 years after treatment discontinuation, but that increases in lumbar spine areal BMD lagged behind that of healthy subjects. We also reported that the effects of treatment discontinuation were more pronounced in growing patients than in those who had achieved final height.

These previous observations were limited to the evaluation of bone mass changes at the lumbar spine and did not provide information on changes in long bones. In the present study we therefore examined the effects of pamidronate discontinuation on the radius using peripheral quantitative computed tomography (pQCT).

**Subjects and methods**

**Subjects**

This observational study comprised patients with a diagnosis of OI type I, III or IV who had received pamidronate at the Shriners Hospital for Children in Montreal. Patients were eligible for pamidronate treatment if they had long-bone deformities or had suffered two or more fractures per year (including vertebral). In the 2 years prior to starting therapy. The present analysis does 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]. The study was approved by the Shriners Hospital Institutional Review Board, and informed written consent was obtained from patients and/or legal guardians, as appropriate.

This study included patients who had received pamidronate for a minimum period of 3 years and had a follow-up period after treatment discontinuation of at least 18 months. All of these subjects had also been included in an earlier report on lumbar spine bone density changes after pamidronate discontinuation [5]. To be part of the present analysis, patients had to be able to undergo pQCT analysis at the two time points of the present analysis. This requires a forearm of sufficient length (at least 18 cm) that is free of metal (such as intramedullary rods). In addition, the patient must be able to hold the arm still for the duration of the measurement (approximately 2 min). Peripheral QCT could not be performed at the lower extremity in these patients, as most had undergone physiotherapy and occupational therapy programs and orthopedic care, as required.

**Anthropometric measurements**

Height was measured using a Harpenden stadiometer (Holtain Limited, Crymlyn, UK). Weight was determined using mechanical scales (Healthometer, Bridgview, USA). Height and weight measurements were converted to age- and sex-specific z-scores based on reference data published by the Centers for Disease Control and Prevention [6]. Forearm length was measured at the non-dominant forearm as the distance between the ulnar styloid process and the olecranon.

**Dual-energy X-ray absorptiometry**

Dual-energy X-ray absorptiometry (DXA) was performed in the anteroposterior direction at the lumbar spine (L1–L4) using a Hologic QDR Discovery device (Hologic Inc., Waltham, MA). An estimate of three-dimensional BMD (unit mg/cm³), commonly called volumetric BMD in the field of bone densitometry, was derived by calculating the ratio between bone mineral content (BMC, the total amount of mineral in the four measured vertebrae) and the extrapolated external volume of the measured bones. This was done as described by Carter et al. [7] using the formula:

\[
\text{Volumetric BMD} = \frac{\text{BMC}}{\text{projection area}^{1.5}}
\]

DXA results were compared to reference data that are based on a study of Canadian children [8].

**Peripheral quantitative computed tomography**

Peripheral QCT was performed at the radius using the Stratec XCT2000® equipment (Stratec Inc., Pforzheim, Germany). Measurements were preferably performed at the non-dominant forearm, but the dominant forearm was analyzed when there was a recent fracture (less than 1 year before the pQCT analysis) on the non-dominant side. Two sites were assessed, representing metaphyseal and diaphyseal bone, respectively, as described [9,10].

For the analysis of the radial metaphysis, the scanner was positioned on the distal forearm and a coronal computed radiograph (scout view) was carried out. The scout view was used to determine the position of a ‘reference line’. In patients with an open growth plate, the reference line was drawn through the most distal portion of the growth plate. When the growth plate was no longer visible, the reference line was drawn through the middle of the ulnar border of the articular cartilage. The measurement was performed at a site whose distance to the ‘reference line’ corresponded to 4% of forearm length (‘4% site’). A single tomographic slice of 2.0 mm thickness was taken at a voxel size of 0.4 × 0.4 × 2 mm. The speed of the translational scan movement was set at 20 mm/s. Image acquisition, processing and the calculation of numerical values were performed using the manufacturer’s software package (version XCT 5.50D). The outer bone contour was detected at the default threshold of 280 mg/cm³. Parameters at that site were calculated using the software’s CALCBD routine.

The radial diaphysis was analyzed at a site whose distance to the ulnar styloid process corresponded to 65% of forearm length (‘65% site’). Parameters for voxel size, slice thickness and scan speed were the same as for the metaphyseal measurement. The cortex of the radial diaphysis was analyzed at a threshold of 710 mg/cm³ using the software’s CORTBD routine. The Strength–Strain Index was determined at a threshold of 480 mg/cm².

The main parameters of pQCT analysis at the radius were BMC (corresponding to the amount of mineral per mm cross-sectional slice thickness), total volumetric BMD (volumetric BMD averaged across the entire bone cross-section), total cross-sectional area (the surface area of the entire bone cross-section, including cortex and marrow space), Strength–Strain Index (an estimate of torsional bone strength), as well as trabecular and cortical volumetric BMD. Results of pQCT analyses were compared to the findings in a reference population of healthy children and adolescents, which has been previously described [9]. This cohort comprised 371 healthy children and...
adolescents aged 6 to 20 years (185 male, 186 female), who participated in an observational study investigating the interrelations of nutrition, growth and metabolism in healthy Caucasian children. Results of pQCT analyses of the present study were transformed to age- and sex-specific z-scores using these reference data [10,11].

Statistical analyses

Statistical analyses were performed on z-scores of densitometric measurements. The expected mean result of these transformed values in a healthy population is 0. The significance of the difference from 0 was calculated by the one-sample t-test. Comparisons between groups were performed using the unpaired t-test. Comparisons between two time points in the same group of subjects were carried out using paired t-tests. 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, USA).

Results

Twenty-three patients (12 female, 11 male; average age 13.4 years, range 5.9 to 21.3 years) were evaluated in this study. These patients had received pamidronate for an average of 5.8 years (range 3.2 to 8.2 years). At the time of the last pamidronate infusion, mean height and weight, lumbar spine BMC, projection area and areal BMD were lower than in healthy individuals of the same age and sex (Table 1). However, volumetric BMD at the spine was close to the age- and sex-specific average for healthy subjects.

Analyses were repeated an average of 1.9 years (range 1.5 to 2.4 years) after the last pamidronate dose had been administered. Lumbar spine densitometry showed a small but significant decline in z-scores for BMC, projection area and areal BMD (Table 1).

Peripheral QCT at the distal radial metaphysis

At the time of the last pamidronate infusion, the average BMC of the distal radial metaphysis was close to the age- and sex-specific mean value for healthy individuals. Total volumetric BMD was slightly elevated (Table 2).

Table 1
Results of anthropometry and lumbar spine DXA in 23 OI patients (age at pamidronate discontinuation 13.4±4.4 years, mean±SD). All results are given as z-scores

<table>
<thead>
<tr>
<th></th>
<th>At PAM stop</th>
<th>At follow-up</th>
<th>Change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>−3.7 (2.7)***</td>
<td>−3.8 (2.7)***</td>
<td>−0.1 (0.4)</td>
<td>0.17</td>
</tr>
<tr>
<td>Weight</td>
<td>−1.1 (1.5)***</td>
<td>−1.0 (1.5)***</td>
<td>+0.1 (0.7)</td>
<td>0.57</td>
</tr>
<tr>
<td>Lumbar spine densitometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC</td>
<td>−1.5 (1.3)***</td>
<td>−1.9 (1.2)***</td>
<td>−0.4 (0.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Projection area</td>
<td>−1.1 (1.2)***</td>
<td>−1.4 (1.4)***</td>
<td>−0.4 (0.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Areal BMD</td>
<td>−1.6 (1.1)***</td>
<td>−2.0 (1.1)***</td>
<td>−0.4 (0.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Volumetric BMD</td>
<td>−0.1 (1.6)</td>
<td>−0.4 (1.8)</td>
<td>−0.4 (1.0)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Values are mean (SD). The significance of the difference to 0 (one-sample t-test) is shown by asterisks: *P<0.05; **P<0.01; ***P<0.001. The P values in the right column represent the significance of the difference between results at the two time points (paired t-test).

PAM: pamidronate.

Table 2
pQCT results at the radial metaphysis (‘4% site’) and the radial diaphysis (‘65% site’)

<table>
<thead>
<tr>
<th></th>
<th>At PAM stop</th>
<th>At follow-up</th>
<th>Changes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metaphysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC</td>
<td>+0.7 (2.1)</td>
<td>−1.7 (1.6)***</td>
<td>−2.4 (2.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total CSA</td>
<td>−0.2 (1.3)</td>
<td>−0.5 (1.4)</td>
<td>−0.4 (0.9)</td>
<td>0.05</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>+1.2 (1.9)**</td>
<td>−1.5 (1.5)***</td>
<td>−2.6 (2.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trabecular vBMD</td>
<td>+0.4 (3.4)</td>
<td>−3.0 (3.6)***</td>
<td>−3.4 (2.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diaphysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC</td>
<td>−1.7 (1.4)***</td>
<td>−2.0 (1.3)***</td>
<td>−0.3 (0.4)</td>
<td>0.005</td>
</tr>
<tr>
<td>Total CSA</td>
<td>−2.9 (1.5)***</td>
<td>−3.1 (1.6)***</td>
<td>−0.1 (0.5)</td>
<td>0.30</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>+1.7 (1.2)***</td>
<td>+1.6 (1.4)***</td>
<td>−0.1 (1.0)</td>
<td>0.63</td>
</tr>
<tr>
<td>Cortical CSA</td>
<td>−1.5 (1.6)***</td>
<td>−1.9 (1.5)***</td>
<td>−0.4 (0.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>Cortical vBMD</td>
<td>+0.8 (1.6)***</td>
<td>+0.9 (1.6)***</td>
<td>0.0 (0.9)</td>
<td>0.86</td>
</tr>
<tr>
<td>Strength–Strain Index</td>
<td>−2.9 (1.6)***</td>
<td>−3.2 (1.6)***</td>
<td>−0.3 (0.6)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

All results are given as z-scores. Values are mean (SD). The significance of the difference to 0 (one-sample t-test) is shown by asterisks: *P<0.05; **P<0.01; ***P<0.001. The P values in the right column represent the significance of the difference between results at the two time points (paired t-test).

PAM: pamidronate; CSA: cross-sectional area; vBMD: volumetric BMD.

These average results masked some large interindividual variations within the patient group (Fig. 1). All but one of the patients below 15 years of age had a positive z-score for BMC, resulting in a mean (SD) z-score of +2.0 (1.0) for this subgroup. In contrast, patients aged 15 years or older had an average BMC z-score of −1.5 (1.5), as the results were negative in all but two

Fig. 1. Results of pQCT analysis at the time of the last pamidronate infusion. Total CSA represents the surface area of the entire radius cross-section, including cortex and marrow space.
of these individuals \( (P < 0.001 \) for the difference between patients below and above 15.0 years of age, \( t \)-test). This variation with age in BMC \( z \)-scores was mirrored by a similar pattern in total volumetric BMD \( z \)-scores, whereas there appeared to be a more steady decline in total bone cross-sectional area \( z \)-scores (Fig. 1).

After pamidronate treatment was stopped, a steep decrease in BMC, total volumetric BMD and trabecular volumetric BMD \( z \)-scores was noted, whereas total bone cross-sectional area \( z \)-scores showed comparatively little change (Table 2). Individual changes varied widely, ranging from a loss of 6.3 SD to a gain of 1.0 SD in BMC \( z \)-score.

As exemplified in Fig. 2, the changes in BMC \( z \)-scores at the site of the metaphyseal pQCT analysis at least partly depend on the growth of the distal radius. Indeed, BMC \( z \)-scores decreased by about 2 SD or more in all patients in whom distal radius growth plates were open when pamidronate was discontinued (Fig. 3). In contrast, none of the 11 patients with closed distal radius growth plates experienced a decrease in metaphyseal BMC \( z \)-score by more than 2 SD.

The more rapid decrease in bone mass after pamidronate discontinuation in the younger subjects eliminated the age-
dependent differences in metaphyseal BMC and total volumetric BMD z-scores that had been present at the time of the last pamidronate infusion (Fig. 4). The average (SD) BMC z-score was now −1.5 (1.3) for patients who had been below 15 years of age at the time of the last pamidronate infusion and −2.1 (1.9) for the older patient group (P=0.34 for the difference between these subgroups, t-test).

Peripheral QCT at the radial diaphysis

At the time of the last pamidronate infusion, average diaphyseal BMC was low, because the bone cross-section was extremely small (Table 2). BMC z-scores decreased with age, which was due to a decrease in bone cross-sectional area z-score with age (Fig. 1). In contrast, total volumetric BMD z-score was positive in most patients, regardless of age.

After pamidronate was discontinued, BMC and cortical cross-sectional area z-scores decreased somewhat, whereas the other measures did not change in a significant manner (Table 2).

Discussion

In this study we found that bone mass and density at the distal radial metaphysis was generally elevated in children and adolescents with OI who had received intravenous pamidronate treatment at a young age. Although no pretreatment results were available, it is likely that these high values were caused by pamidronate therapy. When pamidronate was discontinued, metaphyseal bone mass and density decreased rapidly in growing patients. These changes in the metaphysis were much more pronounced than those at the radial diaphysis or at the lumbar spine. Although pQCT analyses were limited to the radius, it is plausible to assume that results would be similar at the metaphyses and diaphyses, respectively, of other long bones.

The marked differences between metaphysis and diaphysis are probably due to the interaction between the effect of the drug and longitudinal bone growth. As long as growth in length continues, each pamidronate treatment cycle leads to the accumulation of a thin band of mineralized tissue at the interface between growth plate and metaphysis. As growth plate activity continues, these bands become radiographically apparent as metaphyseal lines [12]. Studies in growing rabbits and a case report in a child suggest that these metaphyseal lines resemble transverse trabeculae that consist of a mixture of calcified cartilage and bone tissue, which then gradually remodels into secondary bone [13,14].

In the present study, pQCT analysis at the distal radius was performed in the area of these metaphyseal lines. Consequently, bone mass and density were high in patients who had received the treatment while their radius was still growing. When pamidronate is given after growth plate closure, no metaphyseal lines arise and the treatment effect on metaphyseal bone mass and density is much smaller. It is important to note that the present results reflect the effects of a single drug, pamidronate, given intravenously at a fixed dose every 4 months. Other dosages, other treatment schedules or other bisphosphonates may have different effects.

One might expect that in children who have metaphyseal lines, the pQCT analysis might occasionally exactly overlap with such a line, which conceivably would result in extremely high BMD, similar to cortical BMD. Such high metaphyseal BMD values were not found, however, which may partly be due to the relatively low resolution of the pQCT system. The pQCT measurement beam is 2 mm thick, whereas the thickness of pamidronate-associated transverse trabeculae is probably in the order of 150 μm [14]. Thus, the measurement beam will never capture a “solid” transverse trabecula alone, but will always include some lower density tissue around it. On the other hand, the pQCT beam is unlikely to fall exactly between two metaphyseal lines, because these lines are usually not exactly parallel to the plane of measurement. Thus, the pQCT results at the metaphysis of growing children tend to reflect a mixture of transverse trabeculae and surrounding tissue in most cases.

To appreciate the dynamics of the metaphyseal changes after pamidronate discontinuation, it is important to realize that the metaphyseal site of pQCT analysis is situated approximately 6 to 8 mm proximal to the distal radial growth plate. This growth plate on average adds about 9 mm/year to the length of the radius in growing children [15]. Therefore, the bone tissue that is analyzed by pQCT is about 9 months old in normally growing children, regardless of the chronological age of the patient [15].

In the present study, the second pQCT measurement was performed at least 1.5 years after the discontinuation of pamidronate treatment. As a consequence, the pQCT analysis in growing patients now captured bone tissue that was entirely created after the last pamidronate cycle and therefore was never exposed to the drug. This is very different to the situation in patients whose radius had stopped growing. In such patients, the
same bone tissue was examined at both time points and therefore the changes in bone mass and density were much smaller.

These observations in the radial metaphysis of growing OI patients suggest that previous pamidronate administration has little effect on bone tissue that is created after the last infusion of the drug. This is an important finding, because a widely cited case report had suggested that very high doses of pamidronate might permanently disable the osteoclast system and thus lead to ‘osteopetrosis’ even in bone that was created after the last exposure to pamidronate [16]. In the present study, we found no evidence that this occurs at the doses used at our institution.

Seen from a different perspective, the low metaphyseal bone mass after pamidronate discontinuation suggests that the bone tissue added by growth after the last pamidronate infusion probably is as fragile as the ‘older’ parts of the skeleton were before the start of pamidronate treatment. It is possible that the interface between the dense bone that was exposed to pamidronate and the lesser density bone created after treatment discontinuation are sites at risk of fracture. This was also suggested by the case of a 5-year-old boy who sustained fractures at these locations in two independent minor accidents (Fig. 5). This patient was not part of the study population, as he was too young to collaborate with the pQCT measurement and none of the participants of the present study have sustained similar fractures. Nevertheless, it might be useful to continue administering pamidronate intermittently until growth has stopped, in order to avoid the creation of long segments of ‘untreated’ bone tissue at the ends of long bones. Further studies are necessary to clarify this point.

Compared to the radial metaphysis, post-treatment changes in bone mass and density were much smaller at the radial diaphysis and the lumbar spine. This probably reflects growth-related differences between these skeletal locations. Whereas the metaphyseal measurement in growing patients captures newly created bone only, the other two measurement sites contain a large proportion of ‘older’ bone tissue that therefore was exposed to the drug. The fact that BMC and BMD z-scores decreased somewhat at the radial diaphysis and the lumbar spine after pamidronate discontinuation does not necessarily mean that bone mass was lost at these sites. It is also possible that the densitometric effect of the bone that was exposed to pamidronate was ‘diluted’ by the addition of lower density bone after discontinuation of the drug.

Total volumetric BMD at the radial diaphysis was elevated both at the end of pamidronate therapy and at the follow-up examination. It is unclear to what extent diaphyseal total volumetric BMD is influenced by pamidronate treatment, as similarly high values have been found in OI type I patients who had never received medical therapy [17]. The main reason for high total volumetric BMD values at the diaphysis is that the marrow cavity is very small in most OI patients [17].

In summary, this study shows that the effect of pamidronate discontinuation on long bones is highly dependent on growth. During growth, bone mass and density are often very high in metaphyseal bone that is exposed to pamidronate. When treatment is discontinued, the bone tissue added by longitudinal growth has a much lower density, which may create zones of localized bone weakness. This observation may provide arguments for continuing some form of bisphosphonate treatment for as long as growth continues. However, further studies are needed to determine whether the benefits associated with such an approach outweigh the potential risks of prolonged bisphosphonate administration during growth.

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References


