Osteogenesis imperfecta type VI in childhood and adolescence: Effects of cyclical intravenous pamidronate treatment

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Abstract

Cyclical intravenous treatment with pamidronate is of clinical benefit in children with moderate to severe osteogenesis imperfecta (OI) types I, III and IV, but there is no information on the effects of this treatment on the newly described OI type VI. Here, we report on the results of 3 years of pamidronate treatment in 10 children and adolescents with OI type VI (age range 0.8 to 14.5 years, three girls). Treatment effects were compared to those of 10 patients with OI types I, III, and IV, who were matched for age and disease severity (based on height and lumbar spine areal bone mineral density). During pamidronate therapy, lumbar spine areal bone mineral density z scores increased and lumbar spine vertebral bodies improved in shape. Iliac bone histomorphometry showed a tendency to higher cortical thickness (+53%, P=0.06) but the mineralization defect, a characteristic feature of OI type VI, did not change during pamidronate treatment. Annualized fracture incidence decreased from 3.1 per year before treatment to 1.4 fractures per year during treatment (P<0.05). Regarding mobility, the Pediatric Evaluation of Disability Inventory gross motor score increased by 42% during pamidronate treatment (P<0.005). Significant improvements were also found for age-related z scores of maximal isometric grip force. In comparison to the OI control group, the fracture incidence was higher and the gross motor scores were lower in OI type VI, both before and after pamidronate treatment (P<0.05 for each parameter). No differences were found between the groups for changes in densitometric measures and cortical thickness during pamidronate treatment. Our results suggest that 3 years of intravenous pamidronate therapy led to improvements in bone mineral mass, gross motor function, muscle force and fracture incidence in patients with OI type VI. However, the gains in mobility scores and reductions in fracture incidence during pamidronate treatment are less than in other OI types.

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Introduction

Osteogenesis imperfecta (OI) type VI is a recently described brittle bone disease [1]. OI type VI clinically resembles other forms of moderate to severe OI in that it is characterized by low bone mass, bone fragility and long-bone deformities. However, OI type VI has distinctive findings on bone histology, where a peculiar disorder of bone lamellation (“fish-scale pattern”) and an abundance of unmineralized osteoid are observed. The mineralization of bone tissue appears to be disturbed, even though mineral metabolism is normal. Growth plate mineralization, however, seems to be unaffected, as there are no signs of rickets. Extraskeletal signs that are often present in OI types I to IV, such as blue sclera and tooth involvement, are absent in OI type VI.

OI type VI also seems to have a distinctive genetic basis. In the large majority of patients with OI types I to IV, heritability follows an autosomal dominant pattern and the disease is caused by mutations that affect one of the two genes encoding collagen type I alpha chains (COL1A1 and COL1A2) [2]. In contrast, it appears that OI type VI is inherited in an autosomal recessive fashion and collagen type I mutation studies are negative in all patients that have been studied to date.

Cyclical intravenous treatment with the bisphosphonate pamidronate has a beneficial effect in children and adolescents with moderate to severe forms of OI types I, III and IV [2]. This treatment increases the density and size of lumbar vertebral bodies, decreases fracture rate, and improves muscle force and mobility. However, given the phenotypic and pathogenetic
differences between OI type VI and the other OI types, it is unclear whether pamidronate is also useful in the treatment of OI type VI. In the present study, we therefore assessed the effects of cyclic intravenous pamidronate treatment in pediatric patients with OI type VI. The effects of treatment in this group were compared to those of patients with OI type I, III and IV who had a similar clinical severity of the disease.

Patients and methods

Patients

The study group was comprised of children and adolescents with OI type VI who received cyclical intravenous pamidronate treatment at the Shriners Hospital for Children in Montreal, Canada. As described in detail previously, the diagnosis of OI type VI was made in children who had suffered fractures without adequate trauma and had characteristic findings on iliac bone histology [1]. These were an abnormal pattern of lamellation (the so-called fish-scale pattern) and a large amount of unmineralized osteoid. All of these patients were negative for COL1A1 and COL1A2 mutations [1].

Patients were eligible for pamidronate treatment if they had long-bone deformities or had suffered at least two fractures per year (including vertebrae) during the previous 2 years. All of the 11 patients who were diagnosed with OI type VI at our institution fulfilled these criteria for pamidronate treatment. The parents of one of these patients elected to have the treatment administered at another hospital. The remaining 10 patients were included in the present analysis. Each patient had completed at least 3 years of pamidronate therapy. Pretreatment results in 8 of these patients have been presented before [1]. 4 boys of the study cohort (2 pairs of siblings) were the products of two different families with consanguineous parents. The pedigrees of the two families as well as the genetic aspects of the pattern of inheritance have been discussed elsewhere [1].

Results in OI type VI patients were compared to those in a control group of 10 OI patients who were positive for mutations in either COL1A1 or COL1A2 and who had received at least 3 years of pamidronate treatment at our hospital. One control patient was matched for each patient with OI type VI, based on age and criteria reflecting disease severity at the start of pamidronate therapy (height z score and lumbar spine areal bone mineral density [BMD] z score). Six of the control patients were classified as having OI type IV, one patient had OI type III and three patients were diagnosed with OI type I.

Treatment

Pamidronate was administered intravenously on 3 consecutive days in all patients. As described in detail elsewhere, the timing and dosage of these 3-day cycles varied with age, but the yearly dose of pamidronate remained at 9 mg per kg body weight throughout the study period [2]. Calcium and vitamin D intake were maintained adequate according to the recommended daily allowance. All patients underwent standard physiotherapy, occupational therapy programs, and orthopedic care as required.

Biochemical measurements

Serum total calcium, phosphate and alkaline phosphatase activity were measured using colorimetric methods (Monarch®; Instrumentation Laboratories Inc., Lexington, MA, USA). Serum parathyroid hormone concentration (fragment 39–84) was determined by radioimmunoassay [3]. 25-OH vitamin D and 1,25(OH)2 vitamin D were measured with radioimmunoassays (25-hydroxyvitamin D and 1,25-dihydroxyvitamin D Osteo SP; Incastar Corp., Stillwater, MN, USA). Urinary creatinine concentration was quantified colorimetrically. The bone resorption marker urinary cross-linked N-telopeptide of type I collagen (NTX) was quantified by enzyme-linked immunoabsorbent assay (Osteomark®; Ostex, Seattle, WA) on the second void sample of the morning. Results for urinary NTX/creatinine ratios were compared with published reference data [4]. Patients were fasting at the time of blood and urine sampling.

Radiological analyses

Vertebral morphometry was performed on lumbar vertebra L1 to L4 using lateral spine radiographs that were obtained at baseline and after 2 to 4 years of treatment [5]. Six landmarks were identified at the silhouette of each vertebral body, corresponding to the four corners of the vertebral body and the midpoints of the endplates. The point-to-point distances were measured using a dial reading caliper (General tools, New York, USA) with a precision of 0.1 mm. From these points, anterior, posterior and mid-height as well as lower height were measured. All vertical heights were expressed relative to lower vertebral height (vertebral height ratio). The concavity index for each vertebral body was calculated as the ratio between mid-height and anterior height as described [6].

Dual-energy X-ray absorptiometry was performed in the anterior–posterior direction at the lumbar spine (L1–L4) using a Hologic QDR 2000W or 4500 device (Hologic Inc., Waltham, MA, USA). Areal BMD results were transformed to age-specific z scores combining reference data from Salle et al. and data provided by the densitometer manufacturer [7].

Clinical evaluation

All OI patients were seen several times per year when they received pamidronate treatment. Clinical examination and biochemical measurements were performed at each visit and bone densitometry was performed at least once per year. Information about fractures was retrieved from medical charts. Only

Table 1

<table>
<thead>
<tr>
<th></th>
<th>OI type VI</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (m/f)</td>
<td>10 (7/3)</td>
<td>10 (6/4)</td>
<td>0.99</td>
</tr>
<tr>
<td>Age (years)</td>
<td>7.1 (0.8 to 14.5)</td>
<td>7.1 (0.5 to 13.9)</td>
<td>0.99</td>
</tr>
<tr>
<td>Height (z score)</td>
<td>–3.4 (–9.5 to 0.2)</td>
<td>–3.4 (–7.1 to –1.0)</td>
<td>0.95</td>
</tr>
<tr>
<td>Areal BMD (z score)</td>
<td>–4.6 (–7.1 to 0.29)</td>
<td>–4.6 (–6.4 to –2.4)</td>
<td>0.98</td>
</tr>
<tr>
<td>Serum alkaline phosphatase (U/l)</td>
<td>427 (202)</td>
<td>254 (54)</td>
<td>0.02</td>
</tr>
<tr>
<td>Urinary NTX/creatinine (% of age and sex-specific reference mean)</td>
<td>205 (98)</td>
<td>141 (80)</td>
<td>0.13</td>
</tr>
<tr>
<td>Long bone deformities (n)</td>
<td>6</td>
<td>7</td>
<td>0.60</td>
</tr>
<tr>
<td>Scoliosis (n)</td>
<td>5</td>
<td>5</td>
<td>0.99</td>
</tr>
<tr>
<td>Vertebral compressions (n)</td>
<td>8</td>
<td>10</td>
<td>0.14</td>
</tr>
<tr>
<td>Dentinogenesis imperfecta (n)</td>
<td>0</td>
<td>5</td>
<td>0.01</td>
</tr>
<tr>
<td>Blue sclerae (n)</td>
<td>0</td>
<td>6</td>
<td>0.003</td>
</tr>
<tr>
<td>Wormian bones (n)</td>
<td>0</td>
<td>6</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are given as median (range), mean (SD) or absolute numbers. P values represent the significance of difference between the two groups (unpaired t-test, Mann–Whitney U test or Chi-squared test, as appropriate).
fractures confirmed on radiographs were counted. The annualized fracture incidence was determined separately for the 2 years before the start of pamidronate treatment and the first 3 years thereafter.

Height was measured using a Harpenden stadiometer (Holtain, Crymych, UK). Weight was determined using digital electronic scales for infants and mechanical scales for older children (Healthometer, Bridgeview, IL, USA). 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 [8].

Grip force of the non-dominant hand was determined using a standard adjustable-handle Jamar dynamometer (Preston, Jackson, MI) in patients who were older than 4 years. The maximal value of three trials was noted. Grip force results were transformed to age- and sex-specific z scores using reference data from Rauch et al. [9].

The level of ambulation was scored for each child by physiotherapists experienced in the care of children with bone diseases according to the modified criteria of Bleck [10] using a four-point scale as follows: non-walker, score = 1; therapeutic walker with or without the use of crutches, canes or walker, score = 2; household walker with or without the use of crutches, canes or walker, score = 3; neighborhood or community walker with or without the use of crutches, canes or walker, score = 4. Occupational therapists assessed patients before the start of pamidronate therapy and at each subsequent treatment cycle. The Pediatric Evaluation of Disability Inventory (PEDI) was used to evaluate gross motor abilities [11]. This domain includes 59 mobility items, which were reviewed with questionnaire and observations in each subject. Results are presented as scaled scores. Scaled scores are not adjusted for age and therefore can be used to describe the functional status of children at all ages to document individual improvements over time.

Histomorphometry

Transiliac bone biopsy specimens were obtained at baseline and after 2 to 4 years of pamidronate treatment. Whenever possible, labeling was performed before biopsy using demeclocyclin (15–20 mg/kg per day taken orally for two 2-day periods separated by a 10-day free interval). Transiliac bone samples were collected 4 to 5 days after the labeling using a Bordier trephine from a site located 2 cm below and behind the anterior superior iliac spine. In 8 patients of each patient group, paired biopsy samples were available for histomorphometric analysis. Biopsy preparation and histomorphometric analyses were performed as previously described [12]. Results for histomorphometric parameters were expressed as a percentage of the average results in subjects without metabolic bone disorders [12]. Nomenclature and abbreviations follow the recommendations of the American Society for Bone and Mineral Research [13].

Statistical analyses

Differences between the OI type VI and the control group were tested for significance using unpaired t-test or Mann–Whitney U-test, as appropriate. Paired t-test or Wilcoxon test was used to analyze changes during treatment. The Chi-squared test was used to evaluate differences in frequency of clinical signs between the groups. All tests were two-tailed, and throughout the study, P < 0.05 was considered significant.

Results

Baseline height and lumbar spine areal BMD z scores were low in the OI type VI group and by design were similar to results in controls (Table 1). A similar proportion of patients in both groups had long-bone deformities, scoliosis and vertebral compression fractures. None of the OI type VI patients had blue sclera, dentinogenesis imperfecta or Wormian bones.

Bone and mineral metabolism

At baseline, serum levels of calcium, phosphorus, creatinine, 25 OH vitamin D, 1,25 OH₂ vitamin D, and parathyroid
hormone were within the reference range for all OI type VI patients and the group averages were similar between the OI type VI and the control cohort. However, serum alkaline phosphatase activity was above the upper limit of the reference range (300 U/l) in 6 out of 10 patients with OI type VI (Table 1).

Urinary NTX/creatinine ratios were also clearly above the result expected for age- and sex-matched healthy children.

When OI type VI patients received their first 3-day infusion cycle, average serum calcium dropped by 15% (\(P<0.001\)) but had returned to baseline values by the time the patients were readmitted for the second infusion cycle 2 to 4 months later. This decrease in serum calcium levels was not associated with clinical symptoms in any of the patients. Serum parathyroid hormone levels showed the expected counterregulatory response, with an average increase of 115% during the first infusion cycle (\(P<0.001\)). However, parathyroid hormone levels consistently were back to pretreatment results by the time of the next treatment cycle. The rise in parathyroid hormone was associated with a transient decrease in serum phosphorus (by −38%; \(P<0.001\)) and an increase in 1,25 OH\(_2\) vitamin D levels (by 147%; \(P=0.01\)). These short-term changes in the OI type VI patients were similar to those in the control group (data not shown).

The long-term effects of pamidronate on biochemical results were evaluated in samples that were obtained immediately prior to each treatment cycle. During 3 years of pamidronate treatment, serum levels of calcium, phosphorus, parathyroid hormone and 1,25 OH\(_2\) vitamin D remained stable. However, serum alkaline phosphatase and urinary NTX/creatinine levels decreased continuously during the observation interval (Fig. 1). After 3 years of pamidronate treatment, all of these biochemical results were similar to those of the control group (data not shown).

**Radiology**

None of the OI type VI patients had radiological signs of rickets at any time before or during pamidronate treatment.

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**Fig. 3.** Vertebral body shape in patients with OI type VI and in OI control patients ('OI controls') before and after 2 to 4 years of pamidronate therapy. The heights of each vertebral body are normalized to the lower length of the vertebral body on the lateral lumbar radiograph. Significant treatment changes within the OI type VI group are indicated by asterisks: \(*P<0.05\); \(**P<0.005\). Significant differences (\(P<0.05\)) between controls and OI type VI patients are indicated by \# (for pretreatment results) or \(†\) (for results during treatment).

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**Fig. 4.** Histological changes during pamidronate treatment. (A) Iliac bone specimen from an untreated 2.6-year-old boy with OI type VI. (B) Iliac bone biopsy sample after 2.7 years of pamidronate treatment in the same patient. Cortical thickness had increased from 382 \(\mu\)m to 484 \(\mu\)m. The amount of osteoid (C and D) remained high during pamidronate treatment.
Lumbar spine areal BMD increased considerably during the 3-year treatment period (Fig. 2). The changes in areal BMD during pamidronate treatment were similar to those of controls (mean±SD, +0.26±0.14 g/cm² vs. +0.19±0.16, respectively; \( P = 0.26 \)). Densitometry also showed that the size of the lumbar vertebral bodies increased, as there was a 36% rise in the mean antero-posterior projection area of lumbar vertebral bodies L1 to L4. At the same time, lumbar spine vertebral bodies improved in shape, as determined by vertebral morphometry (Fig. 3). The average anterior, posterior and mid-height of L1 to L4 increased by 13, 15 and 29%, respectively (\( P < 0.01 \)). The mean concavity index of L1 to L4 improved by 14% (\( P < 0.01 \)). However, lumbar vertebral bodies remained more compressed in the OI type VI cohort than in the control group (Fig. 3).

Iliac bone histomorphometry

Iliac bone specimen obtained at baseline documented the mineralization defect in OI type VI patients, with increased osteoid parameters and prolonged mineralization lag time (Fig. 4). Cancellous bone volume was higher and bone formation rate was lower compared to controls both at baseline and during pamidronate treatment. None of the histomorphometric parameters changed significantly during pamidronate treatment, even though there was a trend towards increased cortical width (Fig. 5). The changes in cortical width were numerically higher in the control group, but the difference to the OI type VI cohort did not reach significance.

Effect on growth

During pamidronate treatment, the longitudinal growth of OI type VI patients lagged behind that of healthy children, as shown by a trend towards lower height \( z \) scores (\( −0.7±1.1; \ P = 0.07 \)). The OI type VI group also gained less height during the 3-year follow-up period than the control group (mean±SD, 11.7±5.1 cm vs. 16.6±5.3 cm; \( P = 0.05 \)).

Clinical response

With respect of safety, no major side effects were noted during the 3 years of follow-up. The majority of patients showed an influenza-like reaction accompanied by fever and skeletal pain after their first pamidronate infusion. These symptoms typically arose 12 to 36 h after initiation of the infusion and were controlled with standard antipyretic therapy in all cases.

Prior to the start of pamidronate treatment, fracture rates were higher in patients with OI type VI than in the control group (Table 2). The fracture incidence of OI type VI patients decreased after the start of pamidronate treatment, but still remained higher than in controls (Table 2). The average level of ambulation improved in OI type VI patients during pamidronate therapy but was lower than in controls, both before and after treatment (Table 2). Similarly, gross motor function assessed by the PEDI score improved during pamidronate treatment but was inferior compared to OI controls (Fig. 6). In patients with OI type VI, maximal isometric grip force was low at baseline (\( n = 6; \ z \) score, mean±SD, \( −5.0±2.3 \)) but increased significantly during pamidronate treatment (\( z \) score change, mean±SD, +1.4±1.2).

Discussion

This study provides evidence that young patients with OI type VI benefit from treatment with cyclic intravenous pamidronate. The density and shape of lumbar spine vertebral bodies improved, fractures rates decreased and ambulation levels increased during 3 years of pamidronate treatment. Nevertheless, OI type VI patients experienced more fractures and were less mobile than OI patients in whom the disease was due to mutations affecting collagen type I.

The present results do not directly explain why OI type VI patients had a somewhat less favorable clinical response to pamidronate treatment than control patients. However, it is plausible that differences in treatment response reflect differences in the pathogenetic mechanisms that underlie
these disorders. For example, OI type VI seems to be characterized by extreme bone brittleness. This was highlighted by the difficulty to obtain adequately preserved biopsy specimen for histomorphometric analysis. More objective observations also point to fundamental differences between OI type VI and OI types I, III, and IV. Baseline lumbar spine areal BMD was similarly low in OI type VI and the control group, but OI type VI patients had clearly higher cancellous bone volume in iliac bone samples. This discrepancy between the amount of bone matrix and the amount of mineral is probably explained by the mineralization defect in OI type VI. Thus, a given volume of bone matrix in OI type VI probably contains an abnormally small amount of mineral. The opposite is true in OI types I, III and IV, where the amount of mineral in the organic matrix is elevated [14].

It is unclear whether pamidronate treatment affected the mineralization of the organic matrix, as material bone density was not measured in the present study. However, standard histomorphometric parameters reflecting the mineralization process (osteoid thickness, mineralization lag time) did not suggest a major treatment effect on mineralization, even though the number of informative samples was rather small.

Pamidronate may not have influenced the mineralization defect in OI type VI patients, but lumbar spine areal BMD nevertheless increased markedly. Iliac bone histomorphometry does not satisfactorily explain these densitometric changes, as pamidronate treatment had no effect on cancellous bone volume, and a rather mild (and not statistically significant) effect on cortical bone width. However, areal BMD is also influenced by factors that are not captured by histomorphometry. The density of vertebral bodies increases most at locations that are close to the vertebral end plates [15]. These sclerotic changes may have been an important contribution to the increase in areal BMD. In addition, lumbar spine areal BMD is influenced by vertebral size, which increased during pamidronate treatment.

The observed densitometric changes during pamidronate treatment apparently were associated with clinically relevant improvements in bone strength. This is at least suggested by the vertebral morphometry measurements, which revealed significant reshaping of lumbar vertebral bodies. We also noted improvements in fracture rates and mobility, even though the gains were less in OI type VI patients than in the control group.

Regarding safety, observable adverse events in OI type VI patients were largely limited to the acute phase reaction that also occurs in the majority of patients with other OI types upon first exposure to pamidronate [15,16]. The biochemical fluctuations during the first infusion cycle were also very similar to those reported for other OI types [17,18].

In conclusion, cyclic pamidronate therapy in children and adolescents with OI type VI increases lumbar spine bone density and vertebral shape and appears to improve fracture rates and mobility. The overall treatment effect, however, seems to be somewhat smaller than in OI patients who harbor collagen type I mutations. There clearly is a need for more effective treatment modalities in OI type VI.

Table 2
Clinical response to pamidronate treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at start of treatment (years)</th>
<th>Fracture incidence before treatment (/year)</th>
<th>Fracture incidence during 3 years of treatment (/year)</th>
<th>Ambulation score at start of treatment</th>
<th>Ambulation score after 3 years of treatment</th>
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<tr>
<td>1</td>
<td>14.5</td>
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<td>3</td>
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<td>2</td>
<td>4.2</td>
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<td>0.8</td>
<td>2</td>
<td>4.0</td>
<td>2</td>
<td>4</td>
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<tr>
<td>Mean (SD)</td>
<td>7.1 (4.8)</td>
<td>3.1 (0.5)**</td>
<td>1.4 (0.4)**</td>
<td>2.2 (1.2)**</td>
<td>2.9 (1.2)**</td>
</tr>
</tbody>
</table>

OI controls

| Mean (SD) | 7.1 (4.0)                  | 1.7 (0.5)**                              | 0.6 (0.6)**                                         | 3.3 (0.5)**                          | 3.8 (0.4)**                   |

* Significant difference compared to pretreatment results ($P<0.05$).
** Significant difference between OI type VI and corresponding result of the OI control group ($P<0.05$).

![Fig. 6. Mobility score in OI type VI (Type VI) and OI controls (Control) during 3 years of pamidronate therapy as assessed by PEDI. Significant differences compared to baseline values (Time 0) are indicated by asterisks: *$P<0.05$; **$P<0.01$; ***$P<0.001$. #Significant difference to corresponding mobility score of OI type VI patients ($P<0.05$).]
Acknowledgments

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References