Osteogenesis Imperfecta Types I, III, and IV: Effect of Pamidronate Therapy on Bone and Mineral Metabolism

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Cyclical iv therapy with pamidronate improves the clinical course in children and adolescents with osteogenesis imperfecta (OI). In this study we evaluated the effect of this therapy on bone and mineral metabolism in 165 patients with OI types I, III, and IV (age, 2 wk to 17.9 yr; 86 girls and 79 boys). All patients received iv pamidronate infusions on 3 successive days, administered at age-dependent intervals of 2–4 months. During the 3rd of the first infusion cycle, serum concentrations of ionized calcium dropped by 0.14 ± 0.008 mmol (mean ± SE; P < 0.001), and serum PTH levels transiently almost doubled (P < 0.001). At the same time, urinary excretion of the bone resorption marker type I collagen N-telopeptide related to creatinine (uNTX/uCr) decreased by 61–73% (P < 0.001). Two to 4 months later, ionized calcium had returned to pretreatment levels, and uNTX/uCr remained 30–35% lower than at baseline (P < 0.001). During 4 yr of pamidronate therapy (n = 40 patients), ionized calcium levels remained stable, but PTH levels increased by about 30% (P < 0.01). uNTX/uCr, expressed as a percentage of the age- and sex-specific mean value in healthy children, decreased from 132 ± 13% (mean ± SE) at baseline to 49 ± 3% after 4 yr of therapy (P < 0.001). In conclusion, serum calcium levels can decrease considerably during and after pamidronate infusions, requiring close monitoring especially at the first infusion cycle. In long-term therapy, bone turnover is suppressed to levels lower than those in healthy children. The consequences of chronically low bone turnover in children with OI are unknown at present. (J Clin Endocrinol Metab 88: 986–992, 2003)

These studies provided useful information on the biochemical effects of pamidronate therapy in children with OI, but detailed reports are lacking. This is an important gap in the safety profile of this treatment modality. In the present study we therefore evaluated bone and mineral metabolism in a large group of pediatric OI patients who received pamidronate therapy.

Subjects and Methods

This study comprises patients with a diagnosis of OI type I, III, or IV who received pamidronate therapy at the Shriners Hospital for Children (Montréal, Canada). Patients were eligible for pamidronate treatment if they had long bone deformities or had suffered more than 3 fractures/yr (including vertebrae) during the previous 2 yr (7, 8). This applies to all patients with OI types III and IV and generally to the more severe cases of OI type I. Between October 1992 and January 2002 a total of 165 such patients (age, 2 wk to 17.9 yr; 86 girls and 79 boys) received at least 1 course of pamidronate at our institution. The therapeutic schedule changed at 2.0 and at 3.0 yr of age (see below). For the purpose of the present analysis, the study population was therefore broken down into age groups with identical treatment schedules.

The type of OI was assigned using the Sillence criteria (1). However, some patients fulfilling the Sillence criteria for OI type IV were not included in this group because they could be further classified as having OI type V, VI, or VII on the basis of our expanded classification (10–12). These patients were excluded from the present study. Classification is difficult in patients younger than 3 yr of age. This is especially true when pamidronate therapy is started early in life, because the treatment markedly changes the natural evolution of the disease. Therefore, patients with unclassified OI under 3 yr of age were included in the present study.

Treatment

Pamidronate was administered iv on 3 consecutive days in all patients (Fig. 1). The timing and dosage of these 3-d cycles varied with age.
Results at the start of therapy

<table>
<thead>
<tr>
<th>Reference range</th>
<th>n</th>
<th>&lt;2.0 yr</th>
<th>n</th>
<th>2.0–2.9 yr</th>
<th>n</th>
<th>&gt;3.0 yr</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>Serum</strong></td>
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<tr>
<td>Ca(^{2+}) (mmol/liter)</td>
<td>1.19–1.29</td>
<td>49</td>
<td>1.36 ± 0.008ac,b</td>
<td>14</td>
<td>1.34 ± 0.012b,c</td>
<td>92</td>
<td>1.29 ± 0.004c</td>
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<tr>
<td>P (mmol/liter)</td>
<td>1.23–1.62</td>
<td>50</td>
<td>1.85 ± 0.04ab</td>
<td>15</td>
<td>1.64 ± 0.06ab</td>
<td>84</td>
<td>1.56 ± 0.02ab</td>
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<tr>
<td>25-OH vitamin D (nmol/liter)</td>
<td>34–91</td>
<td>36</td>
<td>79 ± 5b</td>
<td>14</td>
<td>65 ± 6b</td>
<td>89</td>
<td>49 ± 5b</td>
</tr>
<tr>
<td>1,25-(OH)(_2) vitamin D (pmol/liter)</td>
<td>65–134</td>
<td>33</td>
<td>96 ± 11</td>
<td>13</td>
<td>110 ± 20</td>
<td>87</td>
<td>88 ± 5</td>
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<tr>
<td>PTH (pmol/liter)</td>
<td>2.6–10</td>
<td>38</td>
<td>6.6 ± 0.5</td>
<td>15</td>
<td>6.4 ± 0.7</td>
<td>88</td>
<td>7.0 ± 0.3</td>
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<tr>
<td>Alkaline phosphatase (U/liter)</td>
<td>150–300</td>
<td>50</td>
<td>298 ± 12</td>
<td>16</td>
<td>287 ± 24</td>
<td>94</td>
<td>277 ± 8</td>
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<tr>
<td>TRAcP (pmol/min • 1-liter(^{-1}))</td>
<td>3–7</td>
<td>33</td>
<td>8.3 ± 0.4abc</td>
<td>12</td>
<td>5.8 ± 0.5c</td>
<td>86</td>
<td>5.1 ± 0.2c</td>
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<tr>
<td><strong>Urine</strong></td>
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<tr>
<td>uCa/uCr (mmol/mmol)</td>
<td>See Fig. 2</td>
<td>50</td>
<td>1.12 ± 0.11</td>
<td>16</td>
<td>0.84 ± 0.21</td>
<td>95</td>
<td>0.78 ± 0.09</td>
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<tr>
<td>uNTX/uCr (nmol/mmol)</td>
<td>See Fig. 2</td>
<td>49</td>
<td>1196 ± 62abc</td>
<td>16</td>
<td>889 ± 45abc</td>
<td>91</td>
<td>686 ± 34abc</td>
</tr>
</tbody>
</table>

Values are mean ± se. Reference ranges are indicated as established in the authors’ laboratory for children between 4 and 10 yr of age. P values represent the significance of the difference between the three age groups (ANOVA).

a Significantly different compared with patients 2.0 and 2.9 yr of age.

b Significant difference (P < 0.05) compared with patients above 3.0 yr of age.

c Significant difference (P < 0.05) compared with patients less than 2.0 yr of age.

Results

Children less than 2 yr of age received 0.25 mg/kg on the first day of the first cycle, 0.5 mg/kg on d 2 and 3 of the first cycle, and 0.5 mg/kg daily on all 3 d in subsequent cycles. Cycles were repeated every 2 months. Children from 2–3 yr of age received 0.38 mg/kg on the first day of the first cycle, 0.75 mg/kg on d 2 and 3 of the first cycle, and 0.75 mg/kg daily on all 3 d of subsequent cycles. Cycles were repeated every 3 months. Above 3 yr of age, the first 3-d cycle consisted of a dose of 0.5 mg/kg on the first day and 1 mg/kg on d 2 and 3. In subsequent cycles the dose was 1 mg/kg daily for 3 d. Cycles were repeated every 4 months. Thus, the yearly dose of the drug was the same at all ages. Calcium intake was maintained as adequate according to the recommended daily allowance in all patients. All patients underwent physiotherapy and occupational therapy evaluation and support, including exercises and design of special devices for transportation and sitting.

Biochemical measurements

Serum Pi and alkaline phosphatase levels were measured using colorimetric methods (Monarch, Instrumentation Laboratories, Inc., Lexington, MA). Serum ionized Ca (Ca\(^{2+}\)) concentrations were quantified using an electrode method (ICA 2 Ionized Calcium Analyzer, Radiometer, Inc., Copenhagen, Denmark). Serum PTH concentrations (fragment 39–84) were determined by RIA (13). The serum activity of the osteoclast enzyme tartrate-resistant acid phosphatase (TRAcP) was measured with a photometric method (14).

25-Hydroxyvitamin D (25-OH)D and 1,25-dihydroxyvitamin D [1,25-(OH)\(_2\)D] were measured with RIAs (25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D Osteo SP, INCSTAR Corp., Stillwater, MN). uCr and uCa were measured colorimetrically. The bone resorption marker urinary cross-linked N-telopeptide of type I collagen (uNTX) was quantified by ELISA (Osteomark, Ostex, Seattle, WA) on the second void sample of the morning. Patients were fasting at the time of blood and urine sampling.

Statistical analyses

Longitudinal differences between two time points were tested for significance using paired t tests. Differences between more than two time points were tested for significance using ANOVA for repeated measures. Differences between age groups were evaluated using ANOVA. In both types of ANOVA, post hoc comparisons were performed using Bonferroni’s adjustment. All tests were two-tailed, and throughout the study, P < 0.05 was considered significant. These calculations were performed using SPSS software, version 9.0 for Windows (SPSS, Inc., Chicago, IL).

Results

Gender-related differences in biochemical results were found only for the short-term changes in Ca\(^{2+}\) (see below). Therefore, boys and girls were analyzed together in the following analyses.

Baseline results (Table 1)

Serum levels of Ca\(^{2+}\), Pi, 25-OH, and TRAcP were higher in children less than 2 yr of age than in older patients. No significant differences between age groups were found for serum levels of PTH, 1,25-(OH)\(_2\)D, and alkaline phosphatase.

The uCa/uCr ratio was above the age-specific 95th percentile (15) in 41 of the 161 patients (25%) in whom the measurement was performed (Fig. 2). The proportion of patients with a uCa/uCr ratio above the 95th percentile was lower before 3 yr of age (7 of 66, 11%) than after that age (34 of 95, 36%; P < 0.001, by χ\(^2\) test). The uNTX/uCr ratio decreased with age (Fig. 2). Compared with reference results for healthy children (16, 17), uNTX/uCr was significantly decreased in patients under 2 yr (80 ± 5% of the age- and sex-specific mean; P = 0.001 for difference to 100%, by one-sample t test). In contrast, uNTX/uCr was significantly elevated in patients above 3 yr of age (128 ± 6%; P < 0.001).
Changes during the first treatment cycle (Figs. 3–5)

During the first 3 treatment days, serum Ca\(^{2+}\) and Pi levels decreased markedly (Fig. 3). The decrease in Ca\(^{2+}\) was slightly larger (\(P = 0.02\)) in boys (mean ± se, -0.166 ± 0.008 mmol/liter) than in girls (-0.142 ± 0.007 mmol/liter). Even though the youngest patients received lower pamidronate doses, there was no difference between age groups in the decrease in serum Ca\(^{2+}\) and Pi during the first infusion cycle (\(P > 0.15\), by ANOVA). However, as baseline Ca\(^{2+}\) was higher in the infant group (Table 1), the nadirs in Ca\(^{2+}\) levels (Fig. 4) were also higher in the youngest patients (<2.0 yr, 1.22 ± 0.07 mmol/liter; 2.0–2.9 yr, 1.17 ± 0.08 mmol/liter; >3.0 yr, 1.15 ± 0.05 mmol/liter for patients; \(P < 0.001\), by ANOVA). In 24 of the 146 patients with complete data (16%), Ca\(^{2+}\) dropped to levels less than 1.10 mmol/liter on the third day of therapy (Fig. 4). By the time the patients were readmitted for the second treatment cycle, levels had returned to pretreatment results (Fig. 5).

The decrease in Ca\(^{2+}\) during the first 3 d was not associated with serious clinical manifestations, although Chvostek’s sign became positive in many patients. None of the patients required iv Ca substitution during any treatment cycle. The decrease in Ca\(^{2+}\) during the 3 d of pamidronate infusion was higher (\(P < 0.002\), by ANOVA) during the first treatment cycle (-0.14 ± 0.008 mmol/liter) than during the second (-0.10 ± 0.009 mmol/liter) and later treatment cycles (-0.07 ± 0.007 mmol/liter at 1 yr; -0.06 ± 0.007 mmol/liter at 2 yr; -0.02 ± 0.008 mmol/liter at 3 yr; -0.03 ± 0.009 mmol/liter at 4 yr; \(n = 35\) patients with complete datasets).

PTH serum levels increased markedly during the 3 d of the first infusion cycle (Fig. 5). At the start of the second
cycle, PTH was still above baseline levels in patients less than 2.0 yr of age. Similar, but nonsignificant, trends were found in the older age groups. 25-OHD serum concentrations did not change during the first 3 treatment d (Fig. 5). However, in the oldest age group a significant increase above baseline levels was noted when the patients returned for the second infusion cycle. 1,25-(OH)2D levels approximately doubled during the first 3 treatment days and returned to pretreatment values thereafter. Urinary Ca excretion dropped markedly during the first 3 days of therapy. On the first infusion cycle, uCa was undetectable in 54 of the 153 patients (35%) in whom the measurement could be performed. At the start of the second infusion cycle, uCa/uCr was still significantly below baseline levels in the oldest age group.

For markers of bone metabolism, the uNTX/uCr ratio dropped between 61% and 73% during the first 3 treatment days. At the start of the second treatment cycle, uNTX/uCr was still 30–35% lower than at baseline. Changes in TRAcP were less marked and achieved significance only in the oldest age groups. Serum alkaline phosphatase activity decreased between 15% and 20% during the first 3 d of therapy and did not change until the next cycle.

Long-term effects of pamidronate therapy (Fig. 6)

The long-term effects of pamidronate therapy were evaluated in 40 patients who had started therapy between 3.0 and 18 yr of age and had received at least 4 yr of treatment. The number of patients was insufficient for statistical analysis of the younger age groups. The results presented here were analyzed in samples that were obtained just before the start of a new treatment cycle.

There was no long-term change in serum Ca2+, but Pi concentrations decreased with time. Mean PTH levels increased by about 30% during the first 2 treatment yr and then remained stable. At 4 yr after the start of therapy, PTH concentrations were above the reference range in 7 of the 36 patients (19%) in whom this parameter was measured. However, none of these patients had a result that was more than 60% above the upper limit of the reference range.

Serum creatinine levels after 4 yr of pamidronate treatment were similar to pretreatment results (56.3 ± 2.1 vs. 58.5 ± 1.3 μmol/liter; n = 37; P > 0.2; data not shown). Urinary Ca/uCr decreased during the first 2 yr of therapy (P < 0.05). However, results at individual time points were not significantly different from baseline values after adjust-
ment for multiple testing, 25-OHD levels increased in the first treatment year, but thereafter declined steadily. No long-term fluctuations in 1,25-(OH)2D were observed.

uNTX/uCr decreased rapidly during the first year and thereafter continued to decrease more slowly. Expressed as a percentage of the age- and sex-specific mean value in healthy children, uNTX/uCr decreased from 132 ± 13% (mean ± se) at baseline to 49 ± 3% after 4 yr of therapy. In contrast, serum TRAcP activity increased significantly during the first 2 yr and then declined to pretreatment values. Alkaline phosphatase activity decreased steadily throughout the observation period.

Discussion

Pretreatment results

As expected, serum levels of Ca++, Pi, PTH, and vitamin D metabolites were within normal limits for most patients. However, 25-OHD concentrations were in the lower part of the reference range for patients older than 3.0 yr and were clearly lower than those in infants. This may reflect a lower intake of vitamin D-fortified milk in the older patients, possibly combined with a relatively low exposure to sunlight.

uCa/uCr was elevated in a considerable proportion of patients, confirming earlier studies (4–6). This finding may reflect increased calcium excretion, decreased excretion of creatinine, or a combination of both. Low creatinine excretion certainly plays a role, because creatinine is a marker of muscle mass, which is low in children with severe OI (18). Whatever the etiology, the elevated uCa/uCr was not associated with obvious clinical problems in our patients. In accordance with Chines et al. (6), none of our patients had decreased renal function or clinical signs of urolithiasis. However, ultrasound imaging was not performed routinely in the present study, and thus more subtle renal complications may have escaped detection.

Mean uNTX/uCr was increased in patients above 3.0 yr of age, which is similar to the results reported by Brenner et al. (4). A new finding of the present study is that patients less than 2 yr of age had low uNTX/uCr. It is tempting to conclude from this observation that bone resorption activity is low in infants with OI and then increases gradually compared with that in healthy children. However, the ratio between uNTX and uCr not only depends on the activity of bone resorption, but also on bone mass and muscle mass. It

FIG. 6. Long-term effects of pamidronate therapy in patients who were between 3.0 and 18 yr of age at the start of treatment. The shaded areas represent the reference range of parameters that do not vary with age. Significant differences from baseline levels (i.e. time zero) are indicated by asterisks: * , P < 0.05; ** , P < 0.01; *** , P < 0.001.
is difficult to judge which of these factors has a greater influence on the variations with age in uNTX/uCr. In any case, our histomorphometric data show that bone turnover (both formation and resorption) is increased in OI patients between 2 and 14 yr of age (3). It is difficult to obtain bone biopsy samples for histomorphometric analyses in children less than 2 yr of age, but reliable serum markers of bone resorption might be useful alternatives in future studies of bone metabolism in infants with OI.

Short-term effects

Mean serum Ca\(^{2+}\) concentrations decreased rapidly during the first infusion cycle, probably reflecting the diminished calcium influx from bone into the circulating pool. The decrease was similar to that found in adults who received iv pamidronate for Paget’s disease (19). Infants had a similar drop in serum Ca\(^{2+}\), although they received only half as much pamidronate per infusion cycle as older children. This suggests that either infants are more susceptible to pamidronate or that changes in serum Ca\(^{2+}\) are not dose dependent within the dosage range used in this study.

Hypocalcemia was not associated with serious clinical manifestations in our patients. Yet, it should be noted that a high calcium intake was maintained before and during the infusion cycle. It is possible that children with low calcium intake might experience more serious side effects. Our data suggest that sufficient calcium intake and close clinical supervision are especially important during the first infusion cycle, when the hypocalcemic response is most pronounced. In later treatment cycles fluctuations in serum Ca\(^{2+}\) are less marked, which is probably due to a lower bone turnover rate at the start of the infusion.

The decrease in serum Ca\(^{2+}\) was associated with the expected increase in serum levels of PTH and 1,25-(OH)\(_2\)D, and a decrease in uCa/uCr. The intermittent increase in PTH may play a role in the response to treatment, but this remains to be established. The marked decline in serum Pi concentrations probably reflects the combined result of decreased phosphate release from bone and the phosphaturic effect of PTH.

The bone resorption marker uNTX/uCr decreased markedly within the first 3 d of therapy. The magnitude of this decrease was comparable to the usual response of adults who receive pamidronate to treat Paget’s disease (20). Somewhat more surprising is the significant decline in the bone formation parameter alkaline phosphatase within 3 d of therapy. Bone formation is thought to decrease during antiresorptive therapy because antiresorptive therapy reduces remodeling activity (21). However, considering that a remodeling cycle takes many weeks, this mechanism is unlikely to account for a decrease in alkaline phosphatase activity within 3 d. This rapid drop is more compatible with a direct effect of pamidronate on either or both of the two skeletal cells types that express this enzyme, osteoblasts and hypertrophic growth plate chondrocytes (22).

Long-term effects

The long-term effects of pamidronate therapy on mineral metabolism were subtle. PTH levels increased slightly, even though Ca\(^{2+}\) was unchanged. Similar observations have been made in adults who received iv pamidronate for postmenopausal osteoporosis or Paget’s disease (19, 23). The slight elevation in PTH levels may account for the decrease in serum Pi and uCa/uCr, although these changes might also reflect an age-related decline.

25-OHD levels increased after the first treatment cycle in patients above 3.0 yr of age. This may be due to increased vitamin D intake after intensive nutritional counseling at the time of the first treatment cycle. However, this effect was short-lived, as 25-OHD levels started to decline after 1 yr of therapy. Somewhat surprisingly, 1,25-(OH)\(_2\)D levels remained stable in the long-term analysis despite the increase in PTH concentrations.

Pamidronate therapy led to a sustained decrease in the uNTX/uCr ratio to approximately 50% of the age-specific mean value. We have observed a similar decrease in bone turnover by iliac bone histomorphometry (24). A diminished systemic bone resorption activity is thought to be responsible for the therapeutic effect of bisphosphonates in osteopenic disorders (25). Nevertheless, a certain amount of bone turnover may be necessary to repair bone tissue microdamage and thus maintain skeletal integrity (26). Although data are lacking, it appears plausible that microdamage is induced more easily in diseases with abnormal bone matrix, such as OI. It is unknown at present how much bone resorption is needed for microdamage repair in children with OI. At present there is no clinical evidence that pamidronate therapy has a detrimental long-term effect, but this possibility requires close monitoring.

The second bone resorption parameter, serum TRAcP, changed little over time and even showed a paradoxical increase after 2 yr of therapy. Thus, TRAcP does not appear to be a useful marker to follow the effects of pamidronate therapy in children with OI.

In conclusion, cyclical pamidronate therapy in children with OI induces a marked decrease in serum Ca\(^{2+}\), especially during the first treatment cycle. This, however, does not cause clinical problems when calcium intake is sufficient, so that the counterregulatory action of PTH and vitamin D can bring Ca\(^{2+}\) back to normal. Bone turnover decreases within 3 d of therapy and in long-term treatment is suppressed to levels lower than those in healthy children. This is a cause of concern, even though the consequences of chronically low bone turnover in children with OI are unknown at present.

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