Evaluation of a Modified Pamidronate Protocol for the Treatment of Osteogenesis Imperfecta

Telma Palomo1,5 • Maria C. Andrade2 • Barbara S. E. Peters3 • Fernanda A. Reis4 • João Tomás A. Carvalhaes2 • Francis H. Glorieux5 • Frank Rauch5 • Marise Lazaretti-Castro1

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Abstract Intravenous pamidronate is widely used to treat children with osteogenesis imperfecta (OI). In a well-studied protocol (‘standard protocol’), pamidronate is given at a daily dose of 1 mg per kg body weight over 4 h on 3 successive days; infusion cycles are repeated every 4 months. Here, we evaluated renal safety of a simpler protocol for intravenous pamidronate infusions (2 mg per kg body weight given in a single infusion over 2 h, repeated every 4 months; ‘modified protocol’). Results of 18 patients with OI types I, III, or IV treated with the modified protocol for 12 months were compared to 18 historic controls, treated with standard protocol. In the modified protocol, mild transient post-infusion increases in serum creatinine were found during each infusion but after 12 months serum creatinine remained similar from baseline [0.40 mg/dl (SD: 0.13)] to the end of the study [0.41 mg/dl (SD: 0.11)] (P = 0.79). The two protocols led to similar changes in serum creatinine during the first pamidronate infusion [modified protocol: +2 % (SD: 21 %); standard protocol: −3 % (SD: 8 %); P = 0.32]. Areal lumbar spine bone mineral density Z-scores increased from −2.7 (SD: 1.5) to −1.8 (SD: 1.4) with the modified protocol, and from −4.1 (SD: 1.4) to −3.1 (SD: 1.1) with standard protocol (P = 0.68 for group differences in bone density Z-score changes). The modified pamidronate protocol is safe and may have similar effects on bone density as the standard pamidronate protocol. More studies are needed with longer follow-up to prove anti-fracture efficacy.

Keywords Pamidronate • Safety • Renal function • Osteogenesis imperfecta • Efficacy

Introduction

Osteogenesis imperfecta (OI) is a heritable connective tissue disorder that is mainly characterized by bone fragility and low bone mass. Four clinical types of OI are traditionally distinguished [1]. Type I OI comprises patients with mild presentation and normal height, whereas type II OI is lethal in the perinatal period. Type III is the most severe form in children surviving the neonatal period. Patients with a moderate to severe phenotype who do not fit into one of the above categories are classified as type IV OI [1].

Bisphosphonate therapy has been used widely for over 15 years in children with moderate to severe OI and is now considered the standard of care [2]. Cyclical intravenous (IV) therapy with pamidronate infusions is given to increase bone density and decrease fracture rates in children with OI. In the best-studied protocol (‘standard protocol’), pamidronate is given at a dose of 1 mg per kg body weight per day given over 4 h on 3 successive days. These infusion cycles are repeated every 4 months (annual pamidronate dose: 9 mg per kg body weight) [3]. The
drawback of the standard pamidronate protocol is that it puts a significant burden on patients, their caregivers, and health care organizations in terms of time lost from school or work and resource utilization. Besides, this long-term 3-day infusion can be a very relevant issue in countries with reduced number of hospital beds. A shorter pamidronate infusion protocol would improve the compliance on the treatment of children with OI.

Pamidronate is usually infused over an extended period of time due to concerns about renal toxicity involving tubular and glomerular injury. In clinical trials for adults with breast cancer and multiple myeloma, some transient deterioration in renal function occurred in 7–10 % of patients treated with a 2-h infusion of pamidronate [4]. The American Society of Clinical Oncology recommends that pamidronate (90 mg) be infused over at least 2 h [5, 6]. Although the effect of IV bisphosphonates on renal function has not been entirely clarified, nephrotoxicity is a significant potential limiting factor in the use of these agents, and seems to depend on several factors, such as the doses, infusion time, and the type of bisphosphonates [7]. Indeed, most cases of renal deterioration were seen with higher doses, rather than faster infusion time [6]. In a large trial comprising more than 1600 adult patients, the incidence of renal toxicity was 9.3 % for pamidronate and 10.7 % for zoledronic acid [4]. Some published articles suggest that the risk of renal deterioration may not increase significantly when pamidronate is infused over 1 h compared to 2-h infusion [6, 8].

Given these observations, we hypothesized that, if safe and effective, a one-day pamidronate infusion protocol would turn more suitable for the treatment of children with OI. Therefore, in the present study, we evaluated the renal safety of a shorter protocol, with lower annual dose but a higher concentration of pamidronate per dose during an observation period of 1 year.

**Subjects and Methods**

**Subjects**

This study comprised 18 children and adolescents (age: 3–17 years; ten girls, eight boys) with a clinical diagnosis of OI type I (N = 9), III (N = 4), or IV (N = 5), median age of 9.5 years (range 3.4–16.2 years), who were eligible to receive cyclic intravenous pamidronate therapy between May 2011 and June 2013 at the school-hospital of Universidade Federal de São Paulo, Brazil. Patients were eligible if they had clinical signs of OI and long-bone deformities or more than two fractures per year (including vertebrae) during the previous 2 years. The classification of OI types followed the criteria established by Sillence [9]. At enrollment, 14 (78 %) patients had received previous pamidronate treatment and 4 had never received any treatment. The study was approved by the ethics review board of Universidade Federal de São Paulo. Parents or legal guardians gave written informed consent. Assent was obtained from children and adolescents aged 7–17 years. The exclusion criteria were as follows: pre-existing chronic kidney conditions, use of nephrotoxic drugs, and pregnancy at any time of the study.

Results in the modified protocol group were compared to 18 OI patients who received standard protocol pamidronate therapy at the Shriners Hospital for Children (Montréal, Canada) between 1992 and 2002, as described [10]. For the purpose of the present analysis, datasets were selected from the results of this previous study to match with the patients receiving modified protocol by severity, age, sex, and pamidronate treatment status.

**Study Design and Treatment Interventions**

The modified protocol group was a prospective open-label study. Patients received pamidronate infusion cycles every 4 months, for a total of four cycles. Infusion cycles consisted of pamidronate given in a single dose of 2 mg per kg body weight over a 2-h period. The maximum dose of pamidronate in a single infusion was 60 mg. All patients were well hydrated orally before and during every infusion. Each dose was diluted in 10 mL of 0.9 % saline solution per kg body weight and administered over a 2-h period (maximum concentration of 0.2 mg of pamidronate per mL of 0.9 % saline solution). Study visits occurred at baseline, 4, 8, and 12 months of treatment.

Calcium supplementation was maintained as adequate according to the recommended daily allowance in all patients, and vitamin D supplementation was given at a dose of 7000 IU per week. During and after each infusion, blood samples were obtained before the infusion start (T0), and at 4, 8, and 24 h and 7 days later. Bone densitometry and kidney ultrasound were performed at baseline and at 12 months. Heart rate, systolic and diastolic blood pressure were measured at time points T0, 4, and 8 h. Reduction in urine output (documented oliguria <0.5 mL of urine per kg body weight per hour for 8 h) was used as an index of acute renal failure. Urine volume was measured during the first 8 h after each pamidronate infusion.

The historical control group was obtained from the databank of Montreal’s Shriners Hospital for Children. They received pamidronate treatment intravenously at a dose of 1 mg per kg body weight on 3 consecutive days. Each dose was diluted in 0.9 % saline solution and administered slowly over 4 h (maximum concentration 0.1 mg of pamidronate per mL of 0.9 % saline solution). Cycles were repeated every 4 months [10]. Biochemical
profile, including serum total calcium, inorganic phosphorus, and creatinine levels, was performed on each patient at T0, 4, 24, 28, 48, and 52 h at the first infusion. The comparison between modified and standard groups is shown in Table 1.

Anthropometry

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 and adults (FilizolaR Mod E-300, sensitivity of 200 g). Height, weight, and body mass index (BMI) 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].

Radiology

Lumbar spine areal bone mineral density (LS-aBMD) was determined in the anterior–posterior direction at the lumbar spine (L1–L4) using a Hologic QDR 4500 device (Hologic Inc., Waltham, MA). The results were transformed to age-specific Z-scores combining published reference data [12, 13]. X-Ray films of the thoracic and lumbar spine and upper and lower limbs were obtained in all patients from the modified protocol group at baseline and throughout the study when fractures occurred. The postero-anterior radiographs of the left hand were taken, and bone age was determined according to the method of Greulich and Pyle [14].

Biochemical Measurements

After an overnight fast, serum levels of creatinine, blood urea nitrogen, total calcium, phosphorus, sodium, and potassium were measured using standard laboratory methods. The standard method for measurement of creatinine used in both groups was the Jaffé reaction. Urinalysis was determined by reagent strip cells and flow cell digital image, and urine microalbuminuria in an isolated sample was determined by laser turbidimetric method. The estimated glomerular filtration rate was estimated in all patients according to the Schwartz equation [glomerular filtration rate \( \text{mL/(min 1.73 m}^2\) = height (cm) \times constant/serum creatinine (mg/dL)], and the constant was 0.55 (for children \( \geq \) 2 years) [15]. Serum 25-hydroxyvitamin D was quantified by chemiluminescence immunoassay (LIAISON 25 OH Vitamin D Total-Diasorin, Stillwater, MN, USA).

Statistical Analyses

Raw results were transformed to age- and sex-specific Z-scores from the average result in the reference population, using the published reference data cited in the description of each measurement technique.

Mean and standard deviation (SD) were derived for all continuous variables. Longitudinal differences between two time points were tested for significance using paired \( t \) tests. The independent \( t \) test was used to compare the two mean from different groups. Differences between more than two time points were tested for significance using ANOVA for repeated measures. Post hoc comparisons were performed using Bonferroni’s adjustment. All tests were two-tailed, and throughout the study, \( P < 0.05 \) was considered significant. OI severity was coded following the presumed severity (type I = 0, type IV = 1, type III = 2). Statistical analyses were performed utilizing SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA).

Results

A total of 18 OI patients were enrolled in the modified protocol group and 14 of them completed the study. One OI type III patient died from pneumonia 3 months after the third infusion, and 3 others did not receive the last dose of pamidronate because they moved to another state of the country. None of the 14 patients previously treated with pamidronate and of the four patients who had their first infusion during the study had an acute phase reaction after treatment. No other side-effects of the treatment were noted.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison between the standard and modified pamidronate treatment protocols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose [mg/(kg dose)]</td>
<td>2.0</td>
</tr>
<tr>
<td>Final pamidronate concentration in the infusion (mg/ml)</td>
<td>0.2</td>
</tr>
<tr>
<td>Infusion time (h)</td>
<td>2</td>
</tr>
<tr>
<td>Annual dose [mg/(kg year)]</td>
<td>6</td>
</tr>
<tr>
<td>Infusion interval (months)</td>
<td>4</td>
</tr>
</tbody>
</table>
Modified Protocol Group

Mild transient post-infusion increases in serum creatinine were found during each infusion (Fig. 1) but no persistent impairment in the renal function was observed with this treatment schedule during the follow-up period. No significant differences were observed in serum sodium and blood pressure. There was a significant decrease in urea and potassium probably related with oral hydration (Table 2). Mean urine output during the first 8 h of the first infusion was 5.8 (SD: 2.7) ml/(kg h). Urine volume measured during the first 8 h after each pamidronate infusion was >0.5 ml/(kg h) in all patients and infusions.

In the 14 patients from the modified protocol group who completed 12 months of follow-up, serum creatinine remained similar from baseline [0.40 mg/dl (SD: 0.13)] to the end of the study [0.41 mg/dl (SD: 0.11)] (P = 0.79). No significant differences were observed in creatinine clearance, urea, potassium, ionized calcium, and magnesium. There was a significant decrease in microalbuminuria from baseline [6.9 mg/l (SD: 10.1)] to the end of the study [2.6 mg/l (SD: 6.4)] (P < 0.01) (Table 3). Individual results of the 14 OI patients from the modified protocol group show minor fluctuations in serum creatinine from baseline to 7-day after fourth pamidronate infusion (Fig. 2). To determine whether younger patients are more susceptible to increases in serum creatinine after pamidronate treatment, the 18 patients from the modified protocol were divided in 9 younger (median 5.2 years; range 3.4–8.8 years) and 9 older children (median 13 years; range 10.1–16.2 years). No significant differences were observed in serum creatinine in the 9 younger patients during all the four infusions (P = 0.56). The older children had a transient increase in serum creatinine from baseline to 8-h that was recovered from 8-h to 7-day after the pamidronate infusion during the 3rd and 4th infusion (P = 0.02 and P = 0.006, respectively). Serum creatinine remained similar from baseline to the end of the study in younger and older patients from the modified protocol group (P = 0.66 and P = 0.37, respectively). Renal ultrasound showed the kidneys to be normal in size for age, without abnormalities, either at baseline or at the end of the study. In the modified protocol group, 25-hydroxyvitamin D levels increased significantly after 1 year of vitamin D supplementation, from 24 ng/mL (SD: 7) to 30 ng/mL (SD: 8) (P = 0.03). Among the 18 patients, twelve (67 %) did not have any fractures during the 1-year follow-up and six had one long-bone fracture from the time pamidronate therapy was commenced to the last follow-up visit.

Comparison of Protocols

The modified protocol and standard protocol groups were well matched for severity, age, sex, prior pamidronate treatment status, and most other clinical characteristics (Table 4). No clinically significant differences were found between the groups.

Only minor fluctuations in serum creatinine occurred during the first infusion cycle in both groups (Fig. 3). In the modified protocol group, serum creatinine increased by 2 % (SD: 21 %) within 24 h after the start of the first pamidronate infusion, whereas serum creatinine decreased by 3 % (SD: 8 %) in the standard protocol group (P = 0.32). As expected, serum calcium and inorganic phosphorus decreased significantly during the first 24 h in both protocols, nevertheless, with no related symptoms. The changes in serum calcium [MP: −6 % (SD: 6 %); SP: −6 % (SD: 5 %); P = 0.73] and inorganic phosphorus [MP: −10 % (SD: 12 %); SP: −10 % (SD: 15 %); P = 0.91] were similar between groups.

During the 12 months study period, LS-aBMD Z-score increased by 1.1 (SD: 1.1) in the modified protocol group and by 1.0 (SD: 0.6) in the standard protocol group (P = 0.68 for the difference in increase between groups). Changes in height and weight Z-scores were also similar between groups.

Discussion

In this study, we confirmed the main hypothesis that our modified protocol with a shorter and higher concentration per dose of intravenous pamidronate was safe and may have similar effects on LS-aBMD as the standard pamidronate protocol. Intravenous bisphosphonate therapy is widely used to treat bone fragility in children with OI. This is largely based on studies in patients with severe bone

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Fig. 1 Mean serum creatinine levels during and after each pamidronate infusion in patients treated with the modified protocol (n = 18 for infusions 1 to 3; n = 14 patients for infusion 4). The arrows indicate pamidronate infusions. The asterisks indicate a significant difference to the baseline creatinine concentration for the same infusion cycle.
The study showed that transient post-infusion increases in serum creatinine occurred, but no persistent impairment in the renal function was detected during the follow-up period. Renal toxicity of bisphosphonates has been reported in animal studies, and also in adults with multiple myeloma and metastatic breast carcinoma. There are very few data regarding bisphosphonate renal safety in children and teenagers. Previous studies reported transient post-infusion increases in serum creatinine in adults receiving other bisphosphonates, such as intravenous zoledronic acid, ibandronate, and oral risedronate. However, in clinical trials for patients with osteoporosis, studies showed that treatment with these agents did not result in long-term renal function deterioration.

Low creatinine levels are expected in OI patients because creatinine is a marker of muscle mass. Baseline levels of serum creatinine were lower in the modified protocol group than standard protocol group, probably due to different methods, since they were measured in two different laboratories. However, the most relevant aspect we took into consideration was the relative variations in serum creatinine in each group during the pamidronate infusion.

### Table 2

<table>
<thead>
<tr>
<th>N</th>
<th>Baseline</th>
<th>2 h</th>
<th>8 h</th>
<th>24 h</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance [ml/(min 1.73 m²)]</td>
<td>18</td>
<td>168 (32)</td>
<td>179 (72)</td>
<td>148 (29)</td>
<td>168 (40)</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>18</td>
<td>24 (7)</td>
<td>19 (4)</td>
<td>23 (5)</td>
<td>21 (5)</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>18</td>
<td>137 (3)</td>
<td>138 (3)</td>
<td>137 (2)</td>
<td>137 (2)</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>18</td>
<td>4.3 (0.3)</td>
<td>4.2 (0.3)</td>
<td>4.0 (0.3)</td>
<td>4.2 (0.3)</td>
</tr>
<tr>
<td>Microalbuminuria (mg/l)</td>
<td>16</td>
<td>7.4 (9.0)</td>
<td>ND</td>
<td>3.1 (4.2)</td>
<td>ND</td>
</tr>
<tr>
<td>BPsys (mmHg)</td>
<td>18</td>
<td>102.6 (9.6)</td>
<td>101.8 (11.9)</td>
<td>98.7 (15.0)</td>
<td>ND</td>
</tr>
<tr>
<td>BPdias (mmHg)</td>
<td>18</td>
<td>59.6 (9.2)</td>
<td>59 (10.3)</td>
<td>56.9 (10.9)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Results are mean (SD).

**BPsys** systolic blood pressure, **BPdias** diastolic blood pressure, ND not done

P values indicate the significance of the difference between all four time points (ANOVA for repeated measures).

### Table 3

<table>
<thead>
<tr>
<th>N</th>
<th>Baseline</th>
<th>1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>14</td>
<td>0.40 (0.13)</td>
</tr>
<tr>
<td>Creatinine clearance [ml/(min 1.73 m²)]</td>
<td>14</td>
<td>163.50 (29.85)</td>
</tr>
<tr>
<td>Microalbuminuria (mg/l)</td>
<td>14</td>
<td>6.94 (10.18)</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>14</td>
<td>23.36 (6.77)</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>14</td>
<td>4.31 (0.29)</td>
</tr>
</tbody>
</table>

Results are mean (SD). Results at 1 year indicate measures obtained 7 days after the last infusion.
As expected, all patients from both groups showed normal values of serum calcium and inorganic phosphorus. Their concentrations decreased rapidly in both groups during the first infusion cycle, probably reflecting the diminished calcium influx from bone into the circulating pool. Our results are similar to data published previously [3].

The lack of randomized trials comparing drugs and doses in patients with OI makes it difficult to declare the superiority of one regimen over others. In this study, we compared the most widely used protocol (annual dose of 9 mg/kg) [30] with this modified one (annual dose of 6 mg/kg); and our results showed a similar increase in LS-aBMD in both protocols. Due to the small number of study participants and the short follow-up time, a final conclusion on the anti-fracture efficacy of the modified protocol cannot be made. More studies are needed to allow definitive conclusions on efficacy of this new protocol.

Limitations of this study are the relative small number of patients and that is not a randomized study. To overcome this, we included data from historical matched controls. No other more sensitive biomarker measurement for acute kidney injury, such as, cystatin C, or N-acetyl-beta-D-glucosaminidase, was done in addition to serum creatinine. Despite these limitations, this is the first study that compares safety and efficacy between two different protocols for OI. This modified protocol will add greater compliance to OI treatment, either by the convenience of patients and carers or the ability to be run on day-hospital clinics, reducing costs and not requiring hospital beds.

In conclusion, these results suggest that the modified pamidronate protocol was safe and may have similar effects on bone density as the standard pamidronate protocol. Decreasing the infusion time may shorten the waiting list for treatment, reduce the average cost per treatment, and may improve quality of life for the patients, their families, and health care organizations in terms of time lost from school or work and resource utilization. More studies are needed to allow definitive conclusions on efficacy of this new protocol.

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Author Contributions Telma Palomo designed the study, data collection and analysis, preparation of the first draft, conceptualized the project; She is guarantor. Maria C. Andrade, Fernanda A. Reis, João Tomás A. Carvalhaes contributed patient information. Barbara S.E. Peters contributed patient information and helped with statistical analysis of the data. Francis H. Glorieux contributed patient information and revised manuscript content; Frank Rauch and Marise Lazaretti-Castro conceptualized the project, contributed patient information, completed the report and accepts responsibility for the integrity of the data analysis.

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Compliance with Ethical Standards

Conflict of Interest Frank Rauch: Genzyme Inc: Advisory Board member; Novartis Inc: Study grant to institution; Alexion Inc: Study
Human and Animal Rights and Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from study participants or the legal guardians.

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