Published in final edited form as:

Bone. 2007 August ; 41(2): 297–302.

Pamidronate Preserves Bone Mass for At Least Two Years Following Acute Administration for Pediatric Burn Injury

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Abstract

We have previously shown that pamidronate, when given within ten days of burn injury, preserves lumbar spine bone mineral content from admission to discharge in 6-8 weeks and at six months increases both lumbar spine and total body bone mineral content (BMC) over placebo. We followed patients unblinded after 6 months every 3 months up to 2 years post-burn to see if the effects of pamidronate were sustained. Additionally, we assessed bone remodeling at one year post-burn by iliac crest bone biopsy. We enrolled 57 subjects who were initially randomized to pamidronate (n=32) and placebo (n=25). After 2 yr, 21 subjects (pamidronate=8, placebo=13) remained. Analysis of bone densitometry by dual energy x-ray absorptiometry revealed an effect of both treatment ($p<0.012$ for total body BMC, $p<0.001$ for lumbar spine BMC, $p<0.014$ for lumbar spine bone area and $p<0.003$ for lumbar spine bone density (BMD)) and time ($p<0.0003$ on total body BMC, $p<0.001$ on lumbar spine BMC, $p<0.001$ on lumbar spine bone area, and no significant difference on lumbar spine BMD). There was no interaction between treatment and time. Results for bone histomorphometry revealed no effect of treatment on either static or dynamic parameters but did show an effect of time on osteoid area ($p=0.004$, surface $p<0.001$, and width, $p<0.001$). We conclude that acute administration of pamidronate resulted in sustained therapeutic effect on bone and that this type of administration may serve as a useful adjunct to other therapies in the preservation and augmentation of bone mass following severe burns.

Keywords

pamidronate; burns; bone mineral content; bone density

Introduction

Severe burns affect every organ system in the body and burn survivors suffer from a disabling hypermetabolism for approximately one year after burn. Key components of these post-traumatic responses are a significant loss in muscle and bone mass caused by a substantial

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increase of endogenous glucocorticoid secretion and pro-inflammatory cytokine production.^{1,2}

Various mechanisms have been identified as responsible for the significant loss in bone mass in pediatric patients with more than 40% total body surface area (TBSA) burns. Imbalances in hormone and vitamin metabolism such as an eightfold increase in glucocorticoids, hypoparathyroidism resulting in urinary calcium wasting, and a persisting vitamin D deficiency are responsible for a reduction in bone mass as well as bone mineral density in these children.³⁻⁹ The clinical consequences of these alterations are an increased fracture risk, a reduction in growth velocity, and finally, a likely but unproven reduction in peak bone mass.²

Therapeutic approaches have been shown to attenuate and increase bone mass after burn in pediatric patients. Oxandrolone, a synthetic testosterone analogue, and growth hormone demonstrated a significant improvement in bone mass in severely burned children when compared to placebo treatment.^{10,11} However, these drugs were given for nearly one year after burn and required daily injections for growth hormone or pill administration for oxandrolone. Furthermore, some of these therapies are very expensive, especially growth hormone.¹¹

Based on this background, a treatment option during the acute phase, which would only require one or two administrations to prevent bone loss in the first place might be helpful. A recent study by our group investigating the bisphosphonate pamidronate, given within ten days of burn injury showed that lumbar spine bone mineral content (LS-BMC) was significantly higher in the pamidronate group when compared to placebo, and at six months after burn, not only LS-BMC but also total bone mineral content (T-BMC) was significantly increased in the treatment group compared to children receiving placebo.¹² Despite these promising initial results, nothing is known as to whether pamidronate will preserve bone mass during a prolonged recovery period after burn, which might last for up to 24 months.

This study addresses the effects of pamidronate, administered acutely after burn on bone mass of severely burned children for two years after injury.

Methods

Subjects

Severely burned children who had been enrolled in the double-blind randomized, controlled study and were unblinded for analysis for a previous publication¹² were followed from 6 months to 24 months post-burn without further pamidronate administration. Inclusion criteria for entry into the previous study were: age ≤ 19 years, TBSA burns of $\geq 40\%$, and availability for studies during acute hospitalization and at discharge, and at 6, 9, 12, 18, and 24 months after burn. This study was approved by the Institutional Review Board at the University of Texas Medical Branch and Shriners Hospital for Children. Informed written consent was obtained from each patient's guardian with the assent of the child was obtained where appropriate prior to enrollment.

Patients had been randomized to receive intravenously 1.5 mg/kg (maximum dose 90 mg) in 1 liter 5% dextrose and water or an equal volume of saline over 12 hours within 10 days of burn and again 1 week later¹². These unblinded patients were followed at 6, 9, 12, 18 and 24 months post-burn. Patients were examined by physicians including a pediatric endocrinologist and data were reviewed by a pediatric safety committee to screen for adverse side effects such as hypocalcemia.

Bone Mass and Bone Density

The method used to analyze bone mass and density utilizes dual-energy X-ray absorptiometry (DXA) performed using the QDR-4500A absorptiometer (Hologic Inc, Waltham, Mass) with pediatric software. Total bone mineral content (T-BMC), lumbar spine bone mineral content (LS-BMC), lumbar spine bone area (LS-BA), lumbar spine bone mineral density (LS-BMD), as well as lumbar spine bone mineral density Z scores (LS-BMD Z scores) were measured during the acute hospitalization before drug or placebo administration (**baseline**) and at discharge, and at 6, 9, 12, 18, and 24 months after burn. To minimize systematic deviations, the Hologic system was calibrated daily against a spinal phantom in the anteroposterior, lateral, and single-beam modes. Individual pixels were calibrated against a tissue bar phantom to determine whether the pixel was reading bone, fat, lean tissue, or air.

Bone Histomorphometry

The method used to investigate iliac crest bone biopsies has been previously described.¹² Patients received an intravenous dose of doxycycline after pamidronate or placebo was administered within approximately 10 days after burn. The interval between the doxycycline doses was 2 weeks. The patients underwent iliac crest bone biopsies during standard of care operative procedures during the acute hospitalization and at 12 months after burn. At the latter time period, one of the two doses of tetracycline was mailed to the patient advising him or her to take the pills within two weeks of the scheduled reconstructive operation. Goldner trichrome stain and fluorescent microscopy were used to detect changes in static and dynamic parameters.

Statistical Analysis—Data are presented as means $\pm$ SEM. Statistical analysis used two- or three-way ANOVA followed by Tukey's multiple comparison test, when appropriate. Significance was accepted at $p < 0.05$. Student's t-test was used for comparing LS-BMD Z scores at 24 months, with significance accepted at $p < 0.05$. Statistical software (SigmaStat and SigmaPlot, SPSS, Chicago, IL) was used for analyses. For histomorphometric analyses, either Fisher's exact testing or chi-square testing was used.

Results

Demographics

Fifty-seven unblinded patients were followed. The pamidronate group ($n=32$) was slightly larger than the placebo group ($n=25$). The groups did not significantly differ in age, gender, ethnicity, and burn size (Table 1). Fifteen patients in the treatment group (47%) and 13 patients in the placebo group (52%) remained in the study at one year. By the end of the second year 21 patients, 37%, 8 pamidronate (25%) and 13 placebo (52%) were left in the study. None of the patients were lost from the study due to side effects from drug or placebo administration.

Bone Mass and Bone Density

Changes of absolute values are reported as percent change from baseline, or acute values on admission for the burn injury. By three-way ANOVA for treatment, time and age, total body bone mineral content demonstrated a significant treatment effect of pamidronate when compared to placebo, $p < 0.012$ (Figure 1). Additionally, both groups showed a significant increase of T-BMC with time when results of the first year were compared to the increases in the second year after burn, $p < 0.0003$. Children treated with pamidronate showed a positive change in T-BMC from the beginning of the study. In opposition, a positive change in the placebo group was only observed starting 12-18 months after injury. Moreover, while there was a tendency for pamidronate treatment to increase T-BMC at a younger age, less than 13 compared to greater than 13 years of age ($p=0.08$), this tendency never reached significance. Insufficient numbers of females >13 yr old prevented a four-way ANOVA including gender.

Percent changes in LS-BMC were significantly improved with pamidronate treatment when compared to placebo administration 24 months after burn, $p < 0.001$, (Figure 2). The patients in the placebo group have had a negative change in LS-BMC till one year after burn, whereas the pamidronate group was able to increase LS-BMC at all time points. Significant time effects ($p < 0.001$) were also observed within both groups.

Analyzing the percent changes in LS-BA we observed both treatment ($p < 0.014$) and time ($p < 0.001$) effects within both groups when we compared the changes at 24 months to changes seen in the first year of the study (Figure 3).

Lumbar spine bone mineral density was significantly improved with pamidronate administration, $p < 0.003$ (Figure 4). Additionally, a significant time effect was observed within the pamidronate group, which was not seen within the placebo group. At the end of the study period, percent changes in LS-BMD were still negative in the placebo group (-0.7 ± 3.8 SEM). In comparison, patients who had received pamidronate increased their LS-BMD at the end of the investigation (7.8 ± 3.9 , SEM).

Additionally, LS-BMD Z scores were significantly higher pediatric burn survivors who received pamidronate (-0.3 ± 0.5 , SEM, $p < 0.05$) when compared to placebo (-1.6 ± 0.4 , SEM) at 24 months after burn (Figure 5). No significant effect of time was noted within groups.

To determine whether pubertal stage might play a role in the effect of pamidronate on preservation of bone mass, we reviewed the charts of the patient subjects and found that 29 of the 57 (51%) had Tanner staging performed during hospitalization; the pamidronate group had 15 subjects with Tanner staging, 5 of whom were stage 1, and the placebo group had 14 subjects with Tanner staging, 4 of whom were stage 1. Performance of ANOVA failed to demonstrate a significant difference of pamidronate in the pre-pubertal group (Tanner stage 1) compared to the other four stages.

Bone Histomorphometry

Eight patients of the pamidronate group and 8 patients in the placebo received iliac crest bone biopsies during the acute phase and at one year after burn. Patients in either subgroup were not different with regard to their demographics. The histomorphometric analysis revealed no difference due to treatment between groups by Fisher's exact test. Both groups showed a significant reduction in surface osteoblasts only during acute hospitalization and all biopsies showed evidence of improved bone formation ($p < 0.05$) by chi square analysis and increased static osteoid measures at 12 months compared to hospitalization thus demonstrating a positive effect of time, $P < 0.005$ by two-way ANOVA with repeated measures (see Table 2 and Figure 6). It should be noted that because several of the patients received only one tetracycline label at follow-up, improved bone formation was determined by tetracycline uptake in the follow-up biopsy versus no tetracycline uptake during the first biopsy.

Side Effects

No adverse side effects related to the pamidronate, such as hypocalcemia or osteopetrosis, were observed during the study period.

Discussion

In summary, pamidronate, given within the first 10 days after burn is able to preserve bone mass in severely burned children for up to two years until bone formation resumes, approximately 9-12 months after burn.

While pamidronate has not been FDA-approved for use in children, studies in children with bone disorders have revealed a beneficial effect of pamidronate with regard to the several bone disorders. One such disorder is osteogenesis imperfecta, a congenital disorder characterized through mutations in COL1A1 or COL1A2 genes encoding type I collagen, resulting in fragile bones and a significantly increased fracture rate. Although no cure has been established so far, various studies have shown a significant increase in bone area, bone mineral content and density in children with osteogenesis imperfecta treated with pamidronate. Glorieux et al reported their results in thirty children with osteogenesis imperfecta who have been treated with intravenous pamidronate (1.5 or 3.0 mg/kg/day), given for three consecutive days in 4 to 6 month intervals for up to 5 years.¹³ The investigators found a significant improvement in bone mineral density and an improvement in the bone density Z scores. Positive findings were also shown by Arikoski et al.¹⁴ His group demonstrated a significant increase in lumbar spine as well as in total body parameters for bone mineral content, bone mineral density, and bone area. Rauch et al investigated the effects after pamidronate was discontinued for 2 years in children suffering from osteogenesis imperfecta and found that beneficial effects continue.¹⁵ These results of pamidronate treatment of children with osteogenesis imperfecta support of our observations that pamidronate preserves bone mineral content in severely burned children.

Severe adverse events from pamidronate administration have not been observed in our study. Glorieux observed only flu-like events on the second day of the pamidronate infusion cycle and only a slight decrease in serum calcium, which was asymptomatic.¹³ Whyte and colleagues¹⁶ reported osteopetrosis-like lesions with increased bone stiffness and fractures in children receiving a high dose pamidronate therapy including repeated administrations over months.

With regard to our study protocol, we gave pamidronate only twice, separated by a week, without further repetitions. As mentioned in our previous report, we did not observe a significant difference in calcium supplementation for post-burn hypocalcemia between pamidronate and placebo or osteopetrosis-like lesions.¹² We conclude that pamidronate administration limited to the acute hospitalization in severely burned children is a safe therapeutic approach to prevent bone loss.

Although we can demonstrate persisting beneficial effects of pamidronate, we also have to review the limitations of our investigation. Unfortunately, as many of our patients come from great distances for follow-up, we have lost a large number of patients during the follow-up period of 24 months. This was reflected in the wider error bars in both groups as the study proceeded from 12 to 24 months and, along with the apparent recovery of the percentage change from admission to 24 months in the placebo group, the effect on total and lumbar spine bone mineral content at six months compared to the previous study¹² was diluted. However, to our knowledge obtained from other long-term studies there are no differences in the health status between those children who return and those who do not return for follow-up.^{2,5,9-12,17,18} Moreover, the original study had been randomized, which should have eliminated any significant group differences not caused by the treatment. Moreover, despite the high dropout rate, the ANOVA analyses still demonstrated significant effects of treatment.

We conclude, that the acute, non-recurring administration of pamidronate has a beneficial effect in preservation of bone mass until remodeling resumes leaving patients with a bone mass closer to age and sex-matched normals than if patients were not treated.

Acknowledgements

The authors want to thank SM Ott MD for her photomicrographs and for her suggestions for improvement of the manuscript table 2 and D. Benjamin, W. Benjamin, M. Celis, J Huddleston, M. Kelly, and C. Meligro for their valuable technical assistance.

Supported by the National Institutes of Health Grant 1P50 GM 60338, National Institute for Disability and Rehabilitation Research Grant H133A020102-05 and by Shriners Hospital Grant 8640.

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Figure 1.

Percent change in total bone mineral content from baseline to 24 months after burn (d/c: Hospital discharge). Values are means $\pm$ SEM. * Significant difference between placebo and pamidronate, $p<0.05$. # Significant time effect within group when compared to changes at 24 months, $p<0.05$.

Figure 2.

Percent change in lumbar spine bone mineral content (d/c: Hospital discharge). Values are presented as means $\pm$ SEM. * Significant difference between placebo and pamidronate, $p < 0.05$. # Significant time effect within group when compared to changes at 24 months, $p < 0.05$.

Figure 3.

Percent change in lumbar spine bone area from baseline to 24 months after injury (d/c: Hospital discharge). Values are presented as means $\pm$ SEM. * Significant difference between placebo and pamidronate, $p < 0.05$. # Significant time effect within group when compared to changes at 24 months, $p < 0.05$.

Figure 4.

Percent change in lumbar spine bone mineral density when measured by Dual Energy X-ray Absorptiometry (d/c: Hospital discharge). Values are means $\pm$ SEM. * Significant difference between placebo and pamidronate, $p < 0.05$. # Significant time effect within group when compared to changes at 24 months, $p < 0.05$.

Figure 5. Lumbar spine bone mineral density Z scores at 2 years after burn. Note Z scores represent the number of standard deviations from normal, healthy age- and sex-matched children. Values are displayed as means $\pm$ SEM. * Significant difference between placebo and pamidronate, $p < 0.05$.

Figure 6.

Panel (a), labeled “before”, shows a Goldner trichrome stain of the iliac crest at 100X magnification. Note the absence of a significant osteoid seam. Panel (b) shows iliac crest bone biopsy from the same patient at 12 months following burn injury. Note the presence of a long, smooth lamellar seam lined with active osteoblasts. (These photomicrographs were provided courtesy of Susan M. Ott MD, Department of Medicine, University of Washington School of Medicine, Seattle WA).

Table 1

Demographic data of patient population.

	Pamidronate	Placebo
Patients enrolled	32	25
Male/Female ratio	25/7	18/7
Age (years)	12 $\pm$ 3.9	11.5 $\pm$ 3.5
TBSA (%)	61 $\pm$ 14	57 $\pm$ 14
Third-degree burn (%)	44 $\pm$ 25	42 $\pm$ 25

Data are presented as means $\pm$ SD.

Bone Histomorphometry for Children Receiving Pamidronate and Control Acutely and at 12 Months Post-Burn

Table 2

PATIENT	STATIC BONE AREA % total	OSTEOID AREA %bone ar	OSTEOID SURFACE %bone su	ERODED SURFACE %bone su	OSTEOID WIDTH mcm	DYNAMIC TETRACY- UPTAKE Y or N	MAR mcm/d	BFR/TA %/year	DRUG PAM/ CON
HOSPITAL 12m	25.2	0.09	1.5	1.2	2.6	N	0	0	CON
HOSPITAL 12m	30.3	4.2	23.9	0.9	11.6	Y	SINGLE LABEL		
HOSPITAL 12m	33.45	0.22	10.05	11.93	3.37	Y	0.16	0.06	CON
HOSPITAL 12m	46.1	6.5	41.1	16.7	13.3	Y	0.86	6.92	CON
HOSPITAL 12m	22.9	0.17	0.06	1.5	1.8	N	0	0	CON
HOSPITAL 12m	23.4	2.4	31	12.75	4.1	Y	SINGLE LABEL		
HOSPITAL 12m	33.5	0.2	8.4	21.1	3.16	Y	0.33	1.3	CON
HOSPITAL 12m	30.6	2.6	28.7	32.1	10.6	Y	1	34.55	PAM
HOSPITAL 12m	21.5	0.23	9.6	15.3	3.1	N			
HOSPITAL 12m	24.4	2.4	20.9	11.2	6.9	N			
HOSPITAL 12m	30.2	0.5	5.4	4.9	4.8	Y	0.16	0.07	PAM
HOSPITAL 12m	22.1	0.6	12.5	5.8	3.7	Y	SINGLE LABEL		
HOSPITAL 12m	32	0.3	4.3	4.2	2.5	Y	0.36	0.26	PAM
HOSPITAL 12m	23.98	0.56	7.1	4.1	4.6	Y	0.6	5.05	PAM
HOSPITAL 12m	32.9	0.01	0.1	2.8	1.9	N	0	0	PAM
HOSPITAL 12m	20	0.9	35.5	19.7	4.5	Y	SINGLE LABEL		
HOSPITAL 12m	19.4	0.1	5.7	7.3	2.3	Y	0.2	0.07	PAM
HOSPITAL 12m	30.3	2.9	35.7	8.2	8.9	Y	0.83	24.96	CON
HOSPITAL 12m	26.6	0.28	16.8	10.8	3.8	Y	0.27	0.33	CON
HOSPITAL 12m	33.8	1	33.5	6.4	6.3	Y	SINGLE LABEL		
HOSPITAL 12m	30.5	1.8	16.4	14.9	2	Y	0.21	0.35	PAM
HOSPITAL 12m	13.3	2.1	16.9	11	2.1	Y	PATCHY SINGLE LABEL		
HOSPITAL 12m	42.7	2.3	59.6	16.2	6.4	N	0	0	CON
HOSPITAL 12m	10.5	1.1	6.8	7.8	2.8	Y	0.55	10.94	CON
HOSPITAL 12m	26.5	1.6	14.9	9.9	4.9	Y	0.29	0.4	PAM
HOSPITAL 12m	26.7	0.4	8.7	10.6	2.9	N	0	0	PAM
HOSPITAL 12m	CRUSH ARTIFACT					Y	SINGLE LABEL		CON
HOSPITAL 12m	CORTICAL BONE ONLY					Y			
HOSPITAL 12m	FRAGMENTED SAMPLE					Y			
NORMAL RANGE	14.0-35.0	1.0-11.8	32.7 9.0-37.0	0.5 1.0-14.0	9.1 3.9-11.7	Y	SINGLE LABEL 0.5-0.97	3.95-21.08	CON