JOURNAL OF BONE AND MINERAL RESEARCH
Volume 19, Number 7, 2004
Published online on July 1, 2004; doi: 10.1359/JBMR.040303

Clinical Vignette

Sclerotic Metaphyseal Lines in a Child Treated With Pamidronate: Histomorphometric Analysis

Frank Rauch, Rose Travers, Craig Munns, and Francis H Glorieux

CYCLICAL INTRAVENOUS THERAPY with pamidronate is increasingly used to treat children and adolescents with osteogenesis imperfecta (OI) and other osteopenic disorders in children.[1–3] In OI patients, histomorphometric studies of iliac bone samples have shown that this treatment increases cortical thickness and the amount of trabecular bone, but suppresses bone turnover.[4] Typically pamidronate is given in intravenous infusion cycles that are repeated every month to every 4 months.[1–3] As long as growth continues, each of these infusion cycles leaves a dense transverse line in the metaphysis.[1,2,5] The histological nature of these metaphyseal lines has not been elucidated, but it has been speculated that they consist of calcified cartilage and might reduce biomechanical strength.[6]

For obvious reasons, it is difficult to obtain bone tissue from sites where these sclerotic lines are most conspicuous radiographically, i.e., in fast growing long bones. Nevertheless, sclerotic lines are also observed in the iliac wing, which has an apophyseal growth plate (Fig. 1A). This is close to the location where bone biopsy samples are typically obtained for histomorphometric analysis. Although our standard site for transiliac biopsy specimens is 2 cm distal from the apophyseal growth cartilage,[7] growth plate tissue is sometimes inadvertently included in a biopsy specimen. This reduces the value of the specimen for histomorphometric analysis, but offers the opportunity to study sclerotic lines associated with pamidronate treatment.

The present patient was a 12-year-old girl with OI type VII who had received cyclical intravenous pamidronate every 4 months for the past 6.9 years.[8] A biopsy of the left iliac bone was performed after dual tetracycline labeling as part of a follow-up program of long-term pamidronate therapy.[4] The sample was embedded in polymethylmetacrylate, sectioned, and stained with Goldner-Masson Trichrome, as described in detail previously.[7] The entire cancellous bone compartment excluding the endocortical surfaces was analyzed histomorphometrically in three sections that were separated by at least 150 μm, as described previously.[7] Results were compared with age-matched reference data.[7] Trabecular bone volume per tissue volume of the entire cancellous bone compartment was in the upper one-half of the reference range (30.9%; age-specific reference values: 24.4 $\pm$ 4.3%), with normal trabecular thickness (132 μm; reference, 148 $\pm$ 23 μm; Fig. 1B). An identically processed iliac bone sample obtained at the start of pamidronate therapy (6.9 years earlier) had revealed a trabecular bone volume of 12.2% (Fig. 1C). Thus, trabecular bone volume had increased about 2.5-fold during the treatment period.

The bone obtained after pamidronate treatment was arranged in bands parallel to the growth plate (Fig. 1B). The distance between the bands was 1.0–1.3 mm. This was similar to the distance between sclerotic lines in the corresponding radiograph (Fig. 1A). In the band closest to the growth plate, about one-quarter of the mineralized tissue consisted of calcified cartilage (Table 1). The relative amount of cartilage decreased continuously with increasing distance from the growth plate.

Averaged over the entire cancellous compartment, parameters of bone formation were within normal limits (e.g., bone formation rate per bone surface was 52 $\mu m^3/\mu m^2$/year; reference, 37 $\pm$ 17 $\mu m^3/\mu m^2$/year), whereas resorption parameters were elevated (osteoclast surface per bone surface was 2.3%; reference, 0.94 $\pm$ 0.38%; the number of osteoclasts per bone perimeter was 0.82/mm; reference, 0.29 $\pm$ 0.14/mm). As noted in our previous studies, many of the osteoclasts were unusually large, with a high number of nuclei (compare Figs. 1D and 1E).[4]

The increased number of osteoclasts probably reflects the fact that bone turnover is usually elevated close to the growth plate.[7] It is expected that bone formation parameters should be equally elevated at that location, but bone formation rate was within normal limits. This is in accordance with our earlier observation that pamidronate has a more pronounced suppressive effect on histomorphometric parameters of bone formation than on the number of osteoclasts.[4] This lack of treatment effect on osteoclast number may seem paradoxical with an antiresorptive therapy, but pamidronate may exert its effect through interference with

The authors have no conflict of interest.

Genetics Unit, Shriners Hospital for Children, McGill University, Montréal, Québec, Canada.

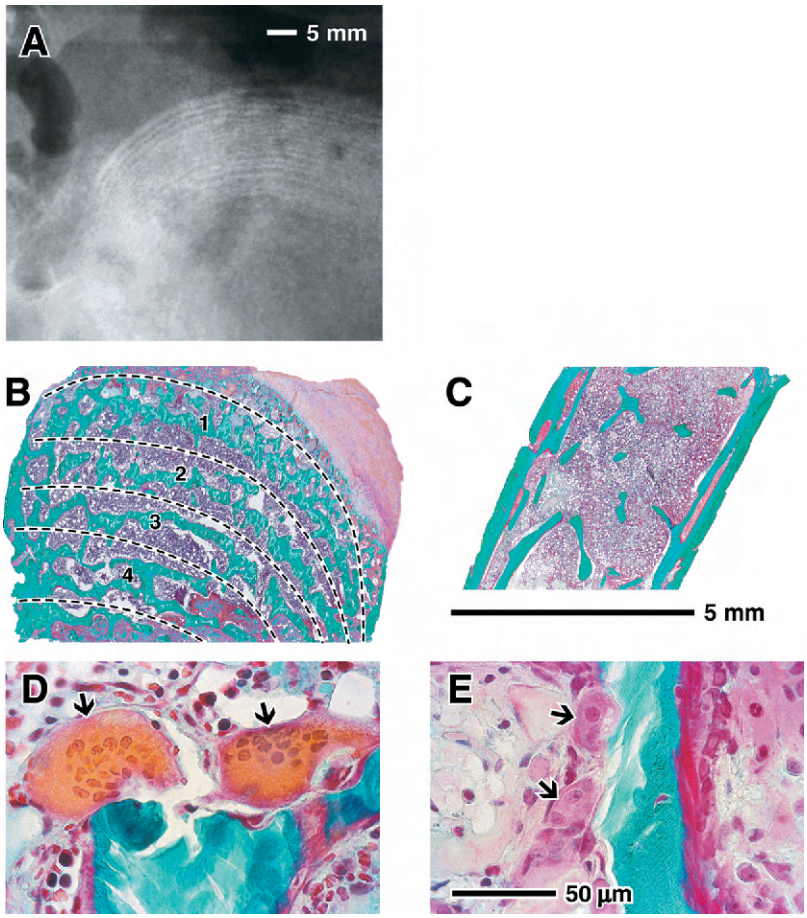

FIG. 1. (A) Radiograph of the left ilium of a 12-year-old girl with OI type VII who had received intravenous pamidronate infusions every 4 months. This radiograph was obtained 4 months before biopsy. (B) Bone biopsy sample of the left ilium obtained after 6.9 years of pamidronate treatment. The numbers indicate bands containing a sclerotic line, corresponding to the lines seen in A. In this Goldner-Masson Trichrome–stained section, mineralized bone is shown in dark green, whereas mineralized cartilage stains light green. (C) Iliac bone sample at the start of pamidronate therapy in the same patient. The magnification in B and C is identical. (D) High magnification view of osteoclasts contained in the section shown in B. These cells were located in the band closest to the growth plate (band 1 in B). (E) High magnification view of osteoclasts from a child with OI type I who had not received bisphosphonates. The location is close to the growth plate, similar to D. The difference in osteoclast color is caused by variability in the staining procedure. The magnification in D and E is identical.

TABLE 1. HISTOMORPHOMETRIC RESULTS OF TRANSILIAC BONE BIOPSY

	Unit	*Band 1*	*Band 2*	*Band 3*	*Band 4*
Cartilage volume/bone volume	%	24.9	16.0	12.3	9.9
Bone formation					
Osteoid thickness	µm	5.0	5.6	5.7	6.6
Osteoid surface/bone surface	%	41.9	31.9	34.4	34.0
Osteoblast surface/bone surface	%	20.3	10.7	13.4	12.1
Mineralizing surface/bone surface	%	22.8	12.4	18.3	16.6
Mineral appositional rate	µm/day	0.81	0.73	0.74	0.84
Bone formation rate/bone surface	$\mu m^3/\mu m^2$/year	68	33	50	51
Bone resorption					
Eroded surface/bone surface	%	30.1	29.6	27.9	21.1
Osteoclast surface/bone surface	%	3.1	2.3	2.1	1.8
Number of osteoclasts/bone perimeter	/mm	1.0	0.8	0.6	0.7

Results are given separately for the bands indicated in Fig. 1B.

osteoclast function rather than by causing osteoclast apoptosis.[9]

In this iliac bone sample, the percentage of calcified cartilage was high in the sclerotic line closest to the growth plate but decreased with increasing distance from the growth plate (Table 1). This suggests that the sclerotic lines seen on radiographs do not represent bars of growth plate cartilage that have been "frozen" by antiresorptive therapy. Rather, they seem to be horizontal trabeculae undergoing turnover. Therefore, new mineralized bone is gradually substituted for the old bone containing calcified cartilage, but the size and shape of these trabeculae are not changed. These are the typical features of the bone remodeling process.[10] The present biopsy specimen did not allow for analysis of sclerotic lines further away from the growth plate, but our previous observations are in accordance with

the view that low remodeling activity may contribute to the persistence of the sclerotic lines during pamidronate treatment.[4]

One might argue that the arrangement of cancellous bone in horizontal trabeculae is not optimal from a biomechanical perspective. In addition, it must be acknowledged that pamidronate does not alter the underlying genetic defect, and therefore, the newly produced bone is likely to have impaired mechanical properties at the material level. Thus, pamidronate does not correct the structural and material deficiencies of OI bone but rather increases its amount.

We speculate that the sclerotic lines that are seen in children during pamidronate treatment arise through similar mechanisms as the transverse lines that occur in the metaphyseal bones of growing children after nonspecific events, such as fever or fractures.[11] These lines are thought to arise after temporary interruption of growth plate activity, when osteoblasts start to deposit bone matrix on the metaphyseal side of the growth plate. When growth resumes, the growth plate moves away from these newly created horizontal trabeculae, which then become visible as so-called "growth arrest lines," or Harris lines, on radiographs.[11] Temporary interruption of growth plate cartilage resorption at the time of a pamidronate infusion may have a similar effect as growth arrest.

ACKNOWLEDGMENTS

We thank Guy Charette and Josée Dépot for technical assistance with biopsy sample processing and Mark Lepik for preparing the figures. This study was supported by the Shriners of North America. CM is a Royal Children's Hospital Foundation/Woolworths Scholar.

REFERENCES

1. Glorieux FH, Bishop NJ, Plotkin H, Chabot G, Lanoue G, Travers R 1998 Cyclic administration of pamidronate in children with severe osteogenesis imperfecta. N Engl J Med **339:**947–952.
2. Astrom E, Soderhall S 2002 Beneficial effect of long term intravenous bisphosphonate treatment of osteogenesis imperfecta. Arch Dis Child **86:**356–364.
3. Henderson RC, Lark RK, Kecskemethy HH, Miller F, Harcke HT, Bachrach SJ 2002 Bisphosphonates to treat osteopenia in children with quadriplegic cerebral palsy: A randomized, placebo-controlled clinical trial. J Pediatr **141:**644–651.
4. Rauch F, Travers R, Plotkin H, Glorieux FH 2002 The effects of intravenous pamidronate on the bone tissue of children and adolescents with osteogenesis imperfecta. J Clin Invest **110:**1293–1299.
5. Grissom LE, Harcke HT 2003 Radiographic features of bisphosphonate therapy in pediatric patients. Pediatr Radiol **33:**226–229.
6. Marini JC 2003 Do bisphosphonates make children's bones better or brittle? N Engl J Med **349:**423–426.
7. Glorieux FH, Travers R, Taylor A, Bowen JR, Rauch F, Norman M, Parfitt AM 2000 Normative data for iliac bone histomorphometry in growing children. Bone **26:**103–109.
8. Ward LM, Rauch F, Travers R, Chabot G, Azouz EM, Lalic L, Roughley PJ, Glorieux FH 2002 Osteogenesis imperfecta type VII: An autosomal recessive form of brittle bone disease. Bone **31:**12–18.
9. Halasy-Nagy JM, Rodan GA, Reszka AA 2001 Inhibition of bone resorption by alendronate and risedronate does not require osteoclast apoptosis. Bone **29:**553–559.
10. Parfitt AM 1990 Bone-forming cells in clinical conditions. In: Hall BK (ed.) The Osteoblast and Osteocyte, vol. 1. Telford Press, Caldwell, NJ, USA, pp. 351–429.
11. Park EA 1964 The imprinting of nutritional disturbances on the growing bone. Pediatrics **33:**815–862.

Address reprint requests to:
Frank Rauch, MD
Genetics Unit
Shriners Hospital for Children
1529 Cedar Avenue
Montréal, Québec H3G 1A6, Canada
E-mail: frauch@shriners.mcgill.ca

Received in original form November 1, 2003; in revised form January 16, 2004; accepted February 26, 2004.