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Bisphosphonate-induced *zebra lines* in fibrous dysplasia of bone: histo-radiographic correlation in a case of McCune Albright-Syndrome

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Abstract

Bisphosphonates (BPs) are currently used in the treatment of diverse bone diseases including fibrous dysplasia of bone (FD). In pediatric patients, a radiographic consequence of cyclical administration of BPs is the development of apo-, epi-, and meta-physeal sclerotic bands, otherwise known as zebra lines, which result from the temporary inhibition of osteoclastic activity at the time of drug treatment. We report here on a child with McCune–Albright syndrome (FD in addition to hyperfunctioning endocrinopathies and skin hyperpigmentation) treated with cyclical intravenous infusions of pamidronate in which conventional radiography, contact microradiography, histology, and backscattered electron image analysis demonstrated that zebra lines formed only where bone was normal, were arrested at the boundary between FD-unaffected and FD-affected bone where bone is sclerotic, and were absent within the undermineralized FD bone. Moreover, in spite of the treatment, the FD lesions continued to expand. This case report is unique because no previously published studies correlated the radiographic and the histologic features of BP-induced zebra lines in the metaphysis of an FD-affected long bone of the limbs.

Keywords

Fibrous dysplasia; McCune–Albright syndrome; Bisphosphonates; Zebra lines; Contact microradiography; BSE-SEM image analysis

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Compliance with ethical standards

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Informed consent: Informed consent was obtained from all individual participants included in the study.

Introduction

Bisphosphonates (BPs) are currently used in the treatment of pediatric patients with low bone density and increased bone fragility in diverse clinical settings including osteogenesis imperfecta, malabsorption syndromes, and neuromuscular disorders [1]. In these conditions, BPs have been demonstrated to reduce the frequency of fractures and the level of pain with significant improvement in the child's quality of life.

When cyclically administered in children, BPs induce the formation of apo-, epi-, and metaphyseal sclerotic bands, otherwise referred to as Bzebra lines[^] [1]. These sclerotic bands are known to be the result of the failure of remodeling of the primary to secondary spongiosa at the physis [2–4] and, in a long bone are oriented perpendicular to the longitudinal axis of the skeletal segment [1, 3, 5, 6]. The age of the patient, the rate of skeletal growth, and the BP dosing regimen are the main determinants of the distance between consecutive bands, which reflects the resumption of osteoclastic activity and the linear growth of bone between consecutive treatments [1, 3, 5, 6].

Based on the anti-resorptive effect of BPs [7] and on the significantly increased osteoclastic activity in the fibrous dysplastic lesional tissue [8, 9], BPs have been extensively used also in fibrous dysplasia (FD) of bone [10–25], a somatic mosaic skeletal disease caused by post-zygotic activating mutations of the α subunit of the stimulatory G protein [26–29] that can be potentially crippling in particular when polyostotic in distribution [30]. However, while a decrease in bone pain and in markers of bone turnover have been almost universally reported, neither a positive effect on the radiographic findings of well-established FD lesions (i.e., refilling or reduction in size or arrest in the expansion) nor prevention of the development of new lesions and fractures has been established conclusively, in particular in pediatric patients [20, 22].

We report here a patient with McCune–Albright syndrome (MAS, OMIM #174800, polyostotic FD along with hyper-functioning endocrinopathies and skin hyperpigmentation) treated with cyclical intravenous infusions of pamidronate who underwent multiple surgical procedures on the long bones of the lower limbs. In one of these procedures, a bone sample that included two zebra lines was obtained, offering the opportunity to provide, for the first time, a detailed correlation of the radiographic and histologic appearance of the zebra lines in the metaphysis of an FD-affected long bone.

Case report

MAS was suspected at another institution in a 2-year-old female presenting with precocious puberty and typical café au lait spots with a Bcoast of Maine[^] profile. At the age of 4, extensive involvement of the right femur was apparent on radiograph. For this reason, a treatment with intravenous pamidronate (1 mg/kg/3-day-cycle every 6 months) was initiated. At the age of 7, the patient was admitted to the Policlinico Tor Vergata where sub-trochanteric and diaphyseal corrective osteotomies stabilized with a cervico-diaphyseal intra-medullary nail were performed. Radiographs performed at this time (Fig. 1a) revealed the characteristic pattern of metaphyseal banding associated with cyclical BP therapy [1, 3–

6]. At the age of 8, because of the progression of the disease, the patient was again admitted to Policlinico Tor Vergata. An intra-medullary nail was placed in the right tibia. Radiographs performed one (Fig. 1b) and two (Fig. 1c) years later revealed newly formed zebra lines in both right and left knee metaphysis. However, in the affected right side, the oldest sclerotic bands were barely detectable, reflecting the progressive expansion of the lesion. In the following years, cyclical pamidronate infusion was continued. At the age of 11, because of severe valgism of the right knee, a new cervicodiaphyseal nail distally blocked with two screws was placed in the right affected femur. Radiographs taken at this time (Fig. 1d) revealed the expected increase in the number of the zebra lines only in the FD-unaffected left knee metaphysis. At follow-up, 1 year later, the patient complained of pain, walked with a marked limp, and radiographs showed a decrease of the right neck-shaft angle to almost 90° and leg-length discrepancy of about 1.5 cm (Fig. 2a). For this reason, it was decided to remove the intra-medullary femoral and tibial nails and to perform proximal femur valgus osteotomy fixed with a screw plate (Fig. 2b and c) and varus osteotomy of the proximal tibia. At the age of 13, distal femoral and proximal tibial epiphysiodesis with eight plates [34] was performed to equalize lower limb length discrepancy. On the radiographs taken at this time (Fig. 1e), zebra lines were virtually undetectable in the right metaphysis, which was completely composed of FD bone.

Bone tissue samples were obtained during the multiple surgical procedures performed on the long bones of the lower limbs. After fixation, they were either routinely processed for paraffin embedding after decalcification (4% EDTA in phosphate buffer, pH 7.0) or processed undecalcified for methyl-methacrylate (MMA) embedding as described previously [8, 29]. The diagnosis of FD of bone was repeatedly confirmed by histology and also by molecular analysis that was performed on genomic DNA extracted from fresh or paraffin embedded tissue as described previously [29, 35, 36] and demonstrated R201C mutation of exon 8 of the GNAS gene (data not shown). Two of the bone samples were obtained at the time of the valgus osteotomy of the proximal femur and varus osteotomy of the proximal tibia (Fig. 2a–c). One of the samples was obtained from the proximal femur (Fig. 2b) and the other from the distal femur where the excision of a bone block was required in order to remove the distal locking screws, which were deeply incarcerated within bone (Fig. 2c). Both samples were processed for MMA embedding and used for contact microradiography (Fig. 2d and e), histology (Fig. 2f) and backscattered electron (BSE) image analysis (Fig. 2g–i). The sample from the proximal femur (Fig. 2d–f, top images) included only FD-affected bone while that from the distal femur (Fig. 2d–f, bottom images) both unaffected- and FD-affected bone. In the FD-unaffected bone, two zebra lines (Fig. 2d–f, bottom images) were unequivocally recognizable. They were the result of an increased amount of trabecular bone and arrested abruptly at the boundary between affected and unaffected bone, where bone formed a trabecular rind-like structure around the FD lesion. Cartilage was focally detected within the bands (Fig. 2g) and within the sclerotic rind (Fig. 2i), indicating their origin from the primary spongiosa. In addition, the level of bone mineralization was lower in FD-affected (Fig. 2h) compared to FD-unaffected bone (Fig. 2g).

Discussion

It is exceptional to have the opportunity to examine bone samples from sites where BP-related zebra lines are most conspicuous radiographically [4, 37]. In our study, a bone sample including BP-related zebra lines was obtained from the affected femur of a patient with MAS. The combination of conventional radiography, contact microradiography, histology, and BSE image analysis unequivocally demonstrated that BP-related zebra lines were not detectable within the FD-affected bone. Specifically, the radiographic time course demonstrated the expected increase in their number only in the FD-unaffected metaphysis. In contrast, in the FD-affected metaphysis, their number progressively reduced, paralleling the expansion of the lesion. When the whole metaphysis was composed of FD, zebra lines were substantially undetectable, indicating that zebra lines develop only where bone is normal and are absent where bone is involved by FD lesions. Contact microradiography, histology, and BSE image analysis demonstrated that zebra lines interrupted at the boundary between FD-unaffected and FD-affected bone, where bone trabeculae were thicker and arranged as a rind around the FD lesion and, in addition, revealed a lower level of bone mineralization in FD-affected compared to FD-unaffected bone.

In principle, zebra lines are not expected to form inside a bone lesion and are expected to disappear when a lesion is growing. Our histo-radiographic correlation study indicates that this is the case in FD of bone in which the fibrodysplastic tissue progressively replaces normal marrow and bone trabeculae, including zebra lines. It is likely that the excess of unmineralized matrix in FD bone trabeculae, previously reported in the literature [8, 29, 30] and confirmed in this study, may contribute to this process. In fact, since BPs selectively absorb to mineral surfaces [7], both the concentration of the drug within the FD bone and its availability in the FD micro-environment at the resumption of osteoclastic activity (that is, in the interval between consecutive infusions) can be expected to be lower compared with normal bone with no significant BP-dependent inhibitory effect on the increased intra-lesional bone remodeling [9]. The absence of changes in the ratio of bone resorption and formation may in turn contribute to explain why BP administration in pediatric patients rarely, if ever, has a beneficial effect (refilling, arrest in expansion, reduction in size) on well-established FD lesions [17, 20, 22–24] and/or a preventive effect on the development of new lesions and fractures [23, 24].

In conclusion, we report a detailed histo-radiographic correlation study of zebra lines in the metaphysis of an FD-affected long bone of the lower limb. Our data suggest that the lack of zebra lines where expected, which reflects the progressive substitution of normal bone with FD bone, could be a useful radiographic parameter to evaluate the expansion of a pre-existing FD lesion or the incipient development of a new one.

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Figure 1.

Radiographic time course showing knee metaphysis during treatment with pamidronate is illustrated in the top panels. Radiographs were obtained 3 (a, age 7), 5 (b, age 9), 6 (c, age 10), 7 (d, age 11), and 9 (e, age 13) years after the initial infusion of the BP. Zebra lines increased progressively in number only in the left FD-unaffected side. At the age of 13 (e), zebra lines were virtually absent in the proximal metaphysis of the right tibia, which was completely composed of FD-affected bone. The diagram in the bottom panel illustrates, at the corresponding time points, the number of the zebra lines in the proximal metaphysis of the left unaffected tibia (black dotted line) and of the right affected tibia (gray continuous line).

Figure 2.

Standing antero-posterior radiograph of the pelvis and femur

1 year after femoral osteotomy and cervico-diaphyseal nail fixation is illustrated in a. The radiographs in b and c illustrate the proximal femur after removal of the nail and valgus osteotomy and the distal femur after the excision of the bone block, respectively. Whole-mount contact microradiographs and Goldner–Masson Trichrome stained histologic sections of the two samples processed for MMA embedding are illustrated in d, e and f, respectively. The sample from the proximal femur (top image in d, e, and f) included FD tissue only. In contrast, both FD-unaffected (N, left side of the images) and FD-affected (FD, right side of the images) bone were evident in the sample from the distal femur (bottom image in d, e, and f). Contact microradiography, performed as described previously [31], is shown in both gray (a: 0 = black = soft tissue and 255 = white = higher mineralization) and pseudo-colored (b: 0 = black = soft tissue and 255 = yellow = higher mineralization) scales, prepared by means of Adobe® Photoshop® CS4 Extended (version 11.0). Both contact microradiography and histology show two zebra lines (one indicated with arrows in d, e, and f) in the FD-unaffected bone and a sclerotic rind (arrowhead in d, e, and f) at the boundary between FD-unaffected and FD-affected bone. Pseudo-colored versions of BSE imaging of one of the two zebra lines, of the FD tissue, and of the sclerotic rind are illustrated in g, h, and i, respectively. BSE signal intensity was calibrated as described previously using thermoset dimethacrylate esters as standards [32, 33]. The color lookup table, which contains eight equal ranges as explained in the inset scale image, demonstrates highly

mineralized cartilage (white signal) within both the zebra line and the sclerotic rind (arrows in g and i). The lower level of mineralization in the FD-affected compared to normal bone is evident in microradiographic, histological, and BSE images. Bar in d–e = 1 mm

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