Basic Science

The Effects of Bone Resorption Inhibitors on the Growth Plate and Proximal Tibial Metaphysis of Rats: Clinical Implications

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ABSTRACT

This study investigated the effects of diphosphonates at scalar doses in a high bone turnover structure, namely, the proximal tibial metaphysis of rats. Arrest of bone modeling was represented by cylindrical-shaped metaphyses, increased height of the perichondrial bone bark, and persistence of primary metaphyseal trabeculae; these changes were dose-related. Higher doses of the inhibitors produced extension of the growth plate and arrest of the mineralization process. The dose-related dissociation between the effects on bone resorption and mineralization allows the therapeutic use of this class of drugs.

Changes induced in bone by resorption inhibitors are evident in high turnover structures such as the growth plate cartilage and metaphyses of long bones. The inverted cone shape of the proximal metaphysis of the rat tibia results from the modeling activity of osteoclasts distributed on the external surface of the cone. Therefore, any alteration of the resorbing activity should appear as a modification of shape and structural organization of the metaphysis and the related structures such as the growth plate cartilage and the peri-chondrial bone bark of Lacroix.¹

In the endochondral ossification process that takes place in the metaphyseal area, a number of phenomena, including hypertrophy of chondrocytes, cartilage matrix calcification, resorption of calcified intercolumnar septa, invasion of capillary buds, and osteogenesis, appear to be closely correlated.²⁻⁵ It is not unexpected that inhibition of resorption can interfere with the other correlated functions of endochondral ossification. This study investigated the effects of calcitonin and diphosphonates, which are known to be inhibitors of bone resorption,⁶⁻⁸ on the metaphyseal complex with particular interest on their relationship to endochondral ossification.

MATERIALS AND METHODS

Sixty-six male Sprague-Dawley rats, with a mean weight of 150 g, were randomly assigned to 5 groups of 12 animals each; an additional group of 6 rats served as a control group.

Group A. These rats received subcutaneous ethane-1-hydroxy-1,1-diphosphonate (EHDP) for 7 and 14 days at the following doses: 10 mg/P/kg/day (milligrams/Phosphorus/kilograms/day) 2 rats, 1 mg/P/kg/day 2 + 2 rats, and 0.1 mg/P/kg/day 2 + 2 rats. 2 + 2 means 2 rats were treated for 7 days and 2 rats were treated for 14 days with the same diphosphonate.

Group B. Rats in this group received subcutaneous 4-amino-1-hydroxy-butan-1,1-diphosphonate (AHBuDP) for 7 and 14 days at the following doses: 10 mg/P/kg/day 2 + 2 rats, 1 mg/P/kg/day 2 + 2 rats, and 0.1 mg/P/kg/day 2 + 2 rats.

Group C. These rats received subcutaneous 4-amino-1-hydroxy-exane-1,1-diphosphonate (AHexDP) for 7 and 14 days at the following doses: 10 mg/P/kg/day 2 + 2 rats, 1 mg/P/kg/day 2 + 2 rats, and 0.1 mg/P/kg/day 2 + 2 rats.

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Fig 1: Photographs demonstrating the inverted-cone-shaped proximal tibial metaphysis in control rats (left) and the cylindrical-shape of the metaphysis due to the arrest of bone modeling after 7 days of treatment with 1 mg/P/kg/day of CI$_2$MDP (right). (Hematoxylin-eosin; original magnification x 20.)

Fig 2: Photograph showing the normal height of the perichondrial bone bark (between arrows) in controls (left). Treatment with 1 mg/P/kg/day of CI$_2$MDP for 7 days increases the bark height because it is not resorbed by osteoclasts at its bottom; the primary metaphyseal trabeculae are not remodeled, giving the metaphysis a denser aspect (right). (Hematoxylin-eosin; original magnification x 50.)

Fig 3: Detail of the bottom of the perichondrial bone bark, where the structure is attacked by osteoclasts. (Hematoxylin-eosin; original magnification x 400.)

Fig 4: Large osteoclasts with an abnormally high number of nuclei are found in the unremodeled metaphyses of CI$_2$MDP treated rats. (Hematoxylin-eosin stain; original magnification x 400.)

Group D. This group received subcutaneous dichloromethylenebisphosphonate (CI$_2$MDP) for 7 and 14 days at the following doses: 10 mg/P/kg/day 2 + 2 rats, 1 mg/P/kg/day 2 + 2 rats, and 0.1 mg/P/kg/day 2 + 2 rats.

Group E. These rats received subcutaneous salmon calcitonin for 7 and 14 days in doses of 5, 50, and 100 U.

Group F. Rats in this group were treated with an equal volume of saline solution injected subcutaneously for 7 (3 rats) and 14 days (3 rats).

Diphosphonates were dissolved in water, and NaOH 0.1 N was added to the solution until the pH was 7.4. Two mL/kg of the neutral solution were injected in each animal, and the same volume of solution was injected in the calcitonin and controls groups.

Thirty mg/kg of tetracycline were injected intraperitoneally at days 0, 7, and 13. The rats were sacrificed after 7 or 14 days of treatment with an overdose of ether. The right and left tibias were dissected from soft tissues and fixed in neutral formalin (10%). The right tibia was decalcified in an EDTA solution for 2 weeks, and the proximal part of the bone was cut in the medial coronal plane and embedded in paraffin. With reference to this plane, sections were prepared with a rotatory microtome and stained with hematoxylin-eosin.

The left tibia was embedded undecalcified in Technovit resin (Kulzer Wehrheim & Co, Germany), and sections of the medial coronal plane were prepared with a cutting-grinding machine. These sections were stained using von Kossa’s method and counterstained with neutral red.

Morphological changes of the proximal metaphysis and growth plate cartilage were classified according to the following criteria: 1) arrest of metaphyseal modeling when the height of the perichondrial bone bark was twice or more than in controls, 2) generalized extension of the growth plate (first or second degree) when the latter was respectively two or more times higher than in controls for the whole width of the plate, and 3) marginal extension of the growth plate when there was a localized area of the growth plate with a height at least twice the remaining part of the plate.

RESULTS

Morphological changes of the growth plate and cartilage of the proximal tibial metaphysis were evident after diphosphonate and calcitonin treatment. The response of bone to each pharmacological treatment was the same, and the observed differences were related more to the dosage than to the type of the drug used.

All of the experimental groups in relation to the dosage of the specific drug and length of administration exhibited a cylindrical shape of the proximal tibial metaphysis, while in control rats (group F), the metaphysis had an inverted-cone shape (Fig 1). Failure of metaphyseal modeling was always associated with an increase of perichondrial bone bark height due to the arrest of osteoclastic resorption of its bottom. The metaphyseal primary trabeculae had a higher density (Fig 2) and were thin, with a calcified core, vertically oriented and densely packed against each other.

Osteoclasts were evidenced by histology in great number on the outer border of the metaphysis and at the bottom of the bark (Fig 3), but they were not active, otherwise there would be no increase of the bark height. Large osteoclasts, with an abnormally high number of nuclei, also were present between
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**Table**

**Metaphyseal and growth plate cartilage changes induced by diphosphonates and salmon calcitonin in rats**

**Abbreviations:** EHDP = ethane-1-hydroxy-1,1-diphosphonate, AHBuDP = 4-amino-1-hydroxy-butane-1,1-diphosphonate, AHexDP = 4-amino-1-hydroxy-exene-1,1-diphosphonate, Cl, MDP = dichloromethylendiphosphonate, and SCT = salmon calcitonin.

*Deceased before the end of the study.*

unremodeled trabeculae (Fig 4).

Despite the arrest of metaphyseal modeling, vascular invasion of the growth plate proceeds normally and the rate of cartilage proliferation at the top equals the erosion rate at the bottom (= growth plate thickness constant). Arrest of metaphyseal modeling in each experimental group was dependent on the dose and time of administration (Table).

Extension of the growth plate cartilage took place only after the arrest of metaphyseal modeling and was characterized by the persistence of hypertrophic chondrocytes and enlargement of the corresponding cartilage layer (Fig 5); these aspects were produced by arrest of vascular invasion from the metaphysis and were associated with the failure of mineralization of the intercolumnar septa (Fig 6), which was limited to the area of extension.

Extension of the growth plate cartilage could be marginal or could involve the whole growth plate (generalized extension) (Fig 5). According to each, where cartilage extended inside the metaphysis, there was disorganization of the hypertrophic cells layer columnar arrangement, whose septa appeared completely uncalcified and collapsed in the lower part of the plate (Fig 7). The synthetic activity of osteoblasts was not significantly affected; around the cartilage core of the metaphyseal primary trabeculae, thick osteoid beams were present. In each experimental group, cartilage extension and mineralization inhibition were dependent on the dosage and time of administration; higher doses or longer times than those
Fig 5: Generalized extension of the growth plate cartilage is evident after 7 days of treatment with 10 mg/P/kg/day of AHBuDP (left). Marginal extension of the growth plate cartilage can be seen after 7 days of treatment with 1 mg/P/kg/day of AIFexDP (right). (Hematoxylin-eosin; original magnification × 20.)

Fig 6: Normal calcification of intercolumnar septa as seen in controls (left). Failure of calcification in Cl₂MDP treated rats is noted when extension of the growth plate occurs (right). (Von Kossa-neutral red; original magnification × 100.)

Fig 7: Extension of the growth plate is characterized by persistence of the hypertrophic cell layer, which is not invaded by metaphyseal vessels. Note the columnar arrangement of chondrocytes is collapsed in the lower part of the plate. (Hematoxylin-eosin; original magnification × 100.)

Fig 8: Modeling of the proximal tibial metaphysis is carried out by the resorbing activity of osteoclasts (arrows). Its arrest alters the density of the proximal metaphyseal trabeculae.

growth plate to extension was not homogeneous and occurred earlier in the outer part than in the center with Cl₂MDP and AIFexDP, while EHDP and AHBuDP produced a more generalized effect. With respect to activity, the more potent diphosphonate was AHBuDP, which at 0.1 mg/P/kg/day showed complete arrest of bone resorption, followed by AIFexDP and Cl₂MDP. However, the wider dissociation between the effect on bone resorption and mineralization was shown by Cl₂MDP.

Calcitonin produced the same changes as diphosphonates, but marginal extension occurred at very high doses (50 and 100 U/day). A generalized effect on the growth plate cartilage was not observed.

**CONCLUSION**

The results of this study indicate that each of the factors tested has the ability to arrest osteoclastic resorption at different doses; however, this ability is always associated with inhibition of the process of mineralization. The former effect is useful for the therapy of several pathologies characterized by loss of bone mass while the latter represents an undesired effect, namely, the risk of osteomalacia in adults and rickets in children. Cases of severe disturbance of mineralization in children receiving EHDP for treatment of myositis ossificans progressiva have been reported, and it is doubtful whether advantages from treatment could compensate for the severe disadvantages.

All diphosphonates showed both effects on resorption and mineralization of calcified tissues; therefore, careful control of the administered doses and monitoring of plasmatic levels is advised to avoid undesired effects on mineralization. Calcitonin did not show the complete arrest of mineralization at the higher dosage used in this study.

**REFERENCES**


