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Epiphyseal Plate Shear Strength in Rats Treated with a Diphosphonate (41009)

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Abstract. Rats treated with a small dose (0.5 mg P/kg/day) of dichloromethylene diphosphonic acid (Cl_2MDP) exhibited a marked increase in the shear strength of the proximal tibial epiphyseal plate with no suppression of longitudinal growth. Our results suggest that mechanisms in addition to the absorption of diphosphonate onto hydroxyapatite crystals account for the increase in epiphyseal plate shear strength noted in our experiment. In this regard, this diphosphonate could be valuable in studies of the normal determinants of epiphyseal plate shear strength. In addition, since Cl_2MDP increased epiphyseal plate strength without suppressing longitudinal growth, the compound may eventually prove to be useful in the treatment of epiphyseal displacements in humans.

The diphosphonates are compounds that are structurally similar to pyrophosphate and possess similar physical chemical properties of inhibiting calcium phosphate precipitation and hydroxyapatite dissolution *in vitro* (1). Several studies have shown that diphosphonates inhibit osteoclastic bone resorption *in vivo* and *in vitro* (2–4).

During the course of our work on the effects of diphosphonate on bone turnover in rats (2), we observed that manual separation of the proximal tibial epiphysis preparatory to metabolic studies was unexpectedly but consistently more difficult in the diphosphonate-treated rats. The extension of this observation resulted in the present study.

Accordingly, herein we describe a novel effect of the diphosphonate dichloromethylene diphosphonic acid (Cl_2MDP): in rats treated with Cl_2MDP, at a dose that did not impede longitudinal growth, the proximal tibia exhibited a marked increase in the shear strength of its epiphyseal plate.

Materials and methods. Twenty weanling male Holtzman rats were separated into control and experimental groups of equal body weight. The animals were housed in individual, suspended cages and fed a semisynthetic diet containing 0.6% calcium and 0.6% phosphorus. The disodium salt of Cl_2MDP was dissolved in water and adjusted to pH 7.4 with sodium hydroxide; the solution was injected subcutaneously once daily (0.5 mg P/kg body wt/day). An equivalent volume of buffered vehicle was administered to the control animals. All animals were injected with tetracycline (15 mg/kg) at the onset of the experiment and weekly thereafter to label bone formed during the experimental period. After 60 days of treatment, the rats were sacrificed by an overdose of ether and the tibiae were removed.

The right tibia from each animal was used for short-beam or shear testing of the proximal tibial epiphysis (5). The specimens were kept moist and were tested fresh within 3 hr of sacrifice. The proximal tibia was carefully mounted in an adjustable constraining jig to assure that the epiphyseal plate was consistently oriented and remained parallel to the applied load. Fine adjustable pins secured the medial and lateral aspects of the proximal tibial epiphysis. Load was applied by a weight drop. Deformation was measured with a linear variable differential transformer; load was recorded by a ring load cell. The load–deformation curve was displayed on a standard storage oscilloscope and photographed. Ultimate load, deformation to failure, area under the loaddeformation curve (energy), and stiffness were determined from the load–deformation curve (5).

The left tibia was used to determine the

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effects of Cl_2MDP treatment on bone length and on parameters of bone turnover by methods described earlier (6, 7). The length of the tibia from the proximal epiphysis to the tibiofibular junction was measured with a caliper. This junction served as the sampling site for a transverse bone section, the microscopic image of which was formed via camera lucida on a digitizing tablet for measurement of bone area, width, and surface parameters. From these data, we calculated rates of osteoid maturation (i.e., conversion of osteoid to a mineralizing matrix), bone formation, and bone resorption (6, 7).

Results. The effects of Cl_2MDP on epiphyseal plate shear strength are shown in Table I. Load to failure was increased by 47%, energy absorption by 54%, and stiffness by 73%, whereas deformation to failure was unchanged (Fig. 1).

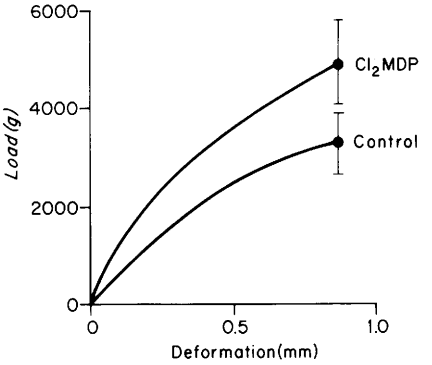

FIG. 1. Load–deformation curves of proximal tibial epiphyseal plates for 6 control and 10 Cl_2MDP-treated rats. Load, stiffness (initial slope of the load–deformation curve), and energy absorption (area under the load–deformation curve), are increased in the Cl_2MDP-treated group.

The decreased load to failure in this control is not attributable to the greater variance in bone length in this group since there was no correlation between bone length and load in data pooled from both groups ($\gamma = 0.34$, $P > 0.05$). The failure site was different in the two groups. In the control bones, failure was entirely confined to the epiphyseal plate, whereas in the test bone failure began in the epiphyseal plate but ended in adjacent metaphyseal bone (Fig. 2). The dose of Cl_2MDP that caused the above changes was sufficient to inhibit endosteal bone turnover, but not longitudinal growth. At the start of the experiment, proximal tibial length was about 9.5 mm and body weight about 60 g. Final body weight and proximal tibial length, both of which increased several-fold during the 60-day test period, were unaffected (see Table I).

Discussion. The normal epiphyseal plate consists of four distinct layers (zones): (I) resting cells, (II) proliferating cells, (III) hypertrophying cells, and (IV) enchondral ossification (3). The intercellular matrix (collagen fibers in an amorphous ground

TABLE I. EFFECTS OF Cl_2MDP ON PARAMETERS OF EPIPHYSEAL PLATE SHEAR STRENGTH, ENDOSTEAL BONE TURNOVER, AND GROWTH

	Control	Cl_2MDP	Percentage Δ	*P*
Epiphyseal plate shear strength				
Load (g)*	3300 ± 593	4840 ± 927	47	<0.005
Energy (N m × 10^{-3})*	14.78 ± 3.22	22.56 ± 7.92	53	<0.001
Stiffness (N/mm)*	39.61 ± 4.37	68.46 ± 8.31	73	<0.001
Deformation (mm)*	0.8 ± 0.1	0.8 ± 0.2	0	NS
Endosteal bone turnover				
Bone formation (mm^3/day)	0.007 ± 0.001	0.005 ± 0.001	−28	<0.001
Bone resorption (mm^3/day)	0.009 ± 0.002	0.006 ± 0.002	−33	<0.025
Osteoid width (μm)	4.8 ± 1.5	5.5 ± 0.7	14	NS
Growth				
Final body weight (g)	278.4 ± 48.8	302.3 ± 27.3	9	NS
Proximal tibial length (mm)**	37.1 ± 2.3	37.1 ± 1.4	—	NS

* $N = 6$ for controls, $\bar{X} \pm$ SD.
** Measured from proximal tibia to tibiofibular junction.

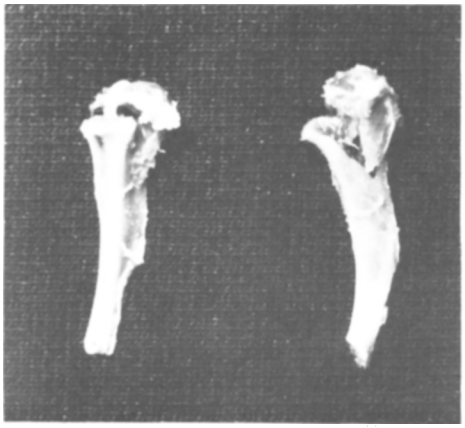

FIG. 2. Proximal tibiae from control rats (left) and Cl_2MDP-treated rats (right) after shear testing to failure. In the control bone, failure was confined to the epiphyseal plate. In the test bone, the plane of failure extended into adjacent bone, as evidenced by the large bone fragment attached to the epiphysis; as a result, a significant portion of the epiphyseal plate remained intact.

substance) provides the "strength," or shear resistance, of the epiphyseal plate (8, 9). The relative ratio of intercellular matrix to cell volume is least in the hypertrophying zone (Zone III) (5, 10–12). Also, the collagen content in this zone is 60% less than that in Zone I (11, 12). Thus, Zone III represents the weakest link in the epiphyseal plate (5, 9–12).

Both shear strength and the width of Zone III, which is a major determinant of the intercellular matrix–cell volume ratio for the plate as a whole, can be influenced by hormone treatment (13). In rats, growth hormone treatment results in a widening of Zone III, an increase in the intercellular matrix–cell volume ratio, and a decrease in shear strength. Conversely, estrogen treatment results in narrowing of Zone III, a decrease in the intercellular matrix–cell volume ratio, and an increase in shear strength (13). Both treatments also alter the major function of the epiphyseal plate, longitudinal growth. Growth hormone causes an increase, and estrogen, a decrease in longitudinal growth (13). Accordingly, longitudinal growth varies directly, and shear strength indirectly, with the width of Zone III. Thus, as would be expected, epiphyseal plate shear strength tends to vary directly with the volume of intercellular matrix within a given periosteal perimeter. Theoretically, qualitative changes in this matrix, such as the concentration of collagen fibrils or the extent of intermolecular collagen crosslinks, could also lead to variation in epiphyseal plate shear strength.

If longitudinal growth had decreased in our study, as was found with estrogen treatment, then the increase in shear strength could reasonably be attributed to an increase in intercellular matrix for a given periosteal perimeter. Although Cl_2MDP in doses much larger than those used in our study inhibited longitudinal growth (14), no such changes occurred in our study (see Table I). Thus, the mechanism of the increase in epiphyseal shear strength in the Cl_2MDP-treated animals differed in some manner from the mechanism in estrogen treatment. The magnitude of the effect of Cl_2MDP on epiphyseal plate shear strength is emphasized by the finding that during failure the cleavage plane was not confined to the epiphyseal plate, but extended to the adjacent bone (Fig. 2). This finding implies that in the epiphyses of the test rats, Zone III was even stronger than some portions of adjacent metaphyseal bone.

Many of the effects of diphosphonates on bone metabolism have been attributed to their physical chemical effects on the mineral phase of bone (15–18). However, such effects would not explain the finding that ethane-1-hydroxy-1,1-diphosphonic acid (EHDP) >> Cl_2MDP inhibits cartilage and bone mineralization *in vivo*, whereas Cl_2MDP >> EHDP inhibits bone resorption *in vivo* (17, 18). Similarly, our finding that Cl_2MDP increased the strength of Zone III, which is not a mineralized tissue, could not be a direct result of an action on the bone mineral phase and thus cannot explain these data.

The present findings of increased epiphyseal shear strength without impairment of longitudinal growth raise a possibility that Cl_2MDP, or a related compound, may ultimately prove effective in the treatment of humans who are predisposed to epiphyseal plate displacement. In this regard, another

diphosphonate, EHDP, in low doses, has been shown to be markedly free of toxic effects in the treatment of patients with Paget's disease of bone (19). While EHDP in large doses may significantly inhibit bone mineralization (17, 18), this effect is not seen with Cl_2MDP (14, 17, 18). Finally, clarification of the mechanism of action of Cl_2MDP may provide a better understanding of the normal determinants of epiphyseal plate shear strength.

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