ALENDRONATE REDUCES FRACTURES WITHOUT INCREASING BONE STRENGTH IN A GROWING MOUSE MODEL OF OSTEOGENESIS IMPERFECTA

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Introduction

Osteogenesis imperfecta (OI) is a heritable disease of the connective tissues caused by heterogeneous mutations in the genes encoding for type I collagen. Clinically diverse, OI is characterized by tissue fragility, skeletal deformities, and in severe cases, death. Recent case studies have reported that bisphosphonates may be effective in reducing fractures and increasing bone density in children with OI {1,2}. To test the hypothesis that bisphosphonates reduce fracture risk and improve bone quality and strength in children with OI, the third-generation bisphosphonate alendronate was evaluated in the growing *oim/oim* mouse, an animal model of moderate-to-severe OI {3}.

Methods

Alendronate (generously supplied by Merck & Co., West Point, PA) or saline alone was administered to 6 week old mice (*oim/oim* and wildtype (+/+) controls, see Table 1 for numbers) via sub-cutaneously implanted Alzet pump (Alza Corp., Pittsburgh, PA) at a dosage of 73 µg alendronate/kg/day for the first 4 weeks and 26 µg alendronate/kg/day for the next 4 weeks under an IACUC-approved protocol. Two mice per group were administered tetracycline to monitor bone formation. At sacrifice, whole body AP radiographs were obtained and digitized, and fractures counted in the long bones and tail. For evaluation of geometrical and density changes, dissected femora were radiographed with a staircase density standard and digitized. To evaluate mechanical and material properties, femora were tested in quasi-static three-point bending using a closed-loop servo-hydraulic test machine (MTS Systems Corporation, Eden Prairie, MN) with Instron electronic controls (Instron Corporation, Canton, MA). Load and displacement data were converted to create stress-strain curves and the Young's Modulus calculated from the elastic region of the curve. Bone ductility was determined as the difference between total strain and yield strain divided by total strain. Differences were considered significant at $p < 0.05$ as determined by the Student's t-test for parametric measures, and by the Mann-Whitney test for counting of fractures.

Results

The average number of fractures sustained by the *oim/oim* mice during the treatment period was significantly reduced for the treated mice, 0.6 vs. 1.8 for the untreated mice. an almost 3-fold reduction. Average radiographic density in the metaphyseal region was significantly increased after treatment in both the *oim/oim* and +/+ mice (Figure 1). In contrast, cortical density was increased for the +/+, but not the *oim/oim* mice.

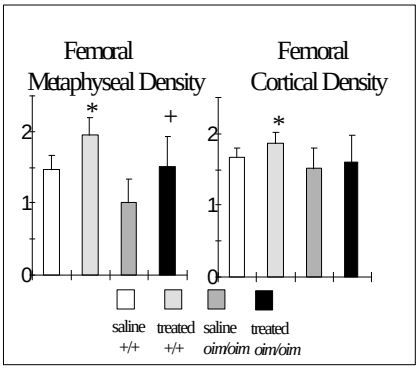

Figure 1. After 8 weeks of alendronate treatment, femoral metaphyseal density significantly increased for the +/+ and *oim/oim* mice. Only the +/+ mice displayed increased cortical density after treatment.
*significantly different from saline +/+
+significantly different from saline *oim/oim*

Although ultimate strength and modulus were increased after treatment for the +/+ femora, no such increase was evident in the *oim/oim* mice (Table 1). In accord with their "brittle" phenotype, bone ductility was significantly reduced for the untreated *oim/oim* compared to the untreated +/+. After treatment, there was no change in ductility of the *oim/oim* bone, but this parameter was significantly reduced in the +/+ animals. Analysis of the tetracycline labeled-bone revealed that 2 to 3 times more cortical bone was formed during the treatment period for the +/+ compared to the *oim/oim* mice.

Table 1. Material Properties of Mice Femora after Alendronate Treatment

	Saline +/+	**Treated +/+**	**Saline *oim/oim***	**Treated *oim/oim***
n	20	16	15	18
Yield Strength (MPa)	72.4 ± 15.8	89.7 ± 16.92	73.8 ± 17.5	66.0 ± 17.0
Ultimate Strength (MPa)	87.4 ± 13.1	103.6 ± 17.6*	77.1 ± 16.9	69.2 ± 16.9
Yield Strain	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01*	0.03 ± 0.01
Total Strain	0.08 ± 0.03	0.06 ± 0.02*	0.04 ± 0.01*	0.03 ± 0.01
Young's Modulus (GPa)	1.76 ± .49	2.45 ± .61.*	2.44 ± 0.65*	2.24 ± 0.70
Percent Ductility	46.4 ± 12.2	32.7 ± 18.3*	8.69 ± 14.8*	9.84 ± 15.5

* Significantly different from untreated +/+

Discussion

This study reveals that although alendronate reduces fractures and increases femoral metaphyseal density in a mouse model of OI, there is no accompanying increase in cortical bone material properties. This is likely attributable to the limited cortical bone formation in the *oim/oim* mice during the treatment period, and is supportive of a mechanism whereby decreased fracture risk is correlated to metaphyseal bone quantity. Overall, the results of this study indicate that alendronate does show promise as an effective therapy for children with OI.

References:
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2. Eur. J. Pediatr., 156:792-794, 1997
3. PNAS, 90:1701-1705, 1993

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