Zoledronate and Raloxifene Combination Therapy Enhances Bone Architecture and Mechanical Properties

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INTRODUCTION

Osteogenesis Imperfecta (OI)

- Bone disorder caused by genetic mutations affecting Type I collagen and characterized by increased bone fragility and fracture risk
- Bisphosphonates only FDA-approved treatment for OI

Raloxifene (RAL)

- FDA-approved agent to treat osteoporosis in postmenopausal women
- Binds to estrogen receptors and increases bone mass by stimulating osteoblasts
- Recent work has shown RAL increases bone hydration by binding to collagen at the collagen/mineral interface [1]

Zoledronate (ZOL)

- Common bisphosphonate used to treat osteoporosis and other bone disorders
- Inhibits resorption by osteoclasts, leading to increases in bone mass and bone mineral density
- Mass-based effects have been seen with OI, however, bone quality has not necessarily improved [2]

HYPOTHESIS

Using the mass-based effects of ZOL in conjunction with the quality improvements seen with RAL, we expect the combination treatment to enhance both the quantity and quality of bone by stimulating different mechanisms.

MATERIALS AND METHODS

Animals and Treatment

- Heterozygous and Wild Type (WT) males from an OI mouse model (Col1a2oim) bred on B6 background
- Subcutaneous injections of RAL (0.5 mg/kg) given 5x/week
- Subcutaneous injections of ZOL (80 µg/kg) given at 8 weeks and 12 weeks
- Control group with no injections
- Treatment began at 8 weeks; mice euthanized at 16 weeks
- Right femurs were harvested, wrapped in saline-soaked gauze, and frozen at -20˚F.

Treatment	WT	Het
Control	4	6
RAL	6	4
ZOL	6	5
RAL+ZOL	6	4

Table 1. Number of mice per group for each genotype.

Microcomputed Tomography (µCT)

- Right femurs were scanned using a Skyscan 1172 at 10-micron resolution
- Scans were reconstructed into a 3D model with nRecon and rotated to a consistent orientation with DataViewer
- Trabecular regions at distal femur were analyzed with CTan; cortical regions at mid-diaphysis with Matlab

Mechanical Testing

- 3-point bending (8 mm support span with load at half the span)
- Tested with anterior surface in tension

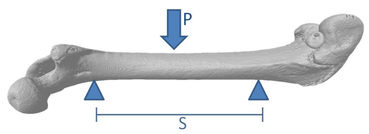

Figure 1. Setup for mechanical testing with 3-point bending technique.

Statistical Analysis

- Statistically analyzed the effects of each treatment versus control in each genotype
- One Way ANOVA with Post-hoc Dunnett's tests to compare to the control

RESULTS AND DISCUSSION

Bone Microarchitecture

Trabecular Properties

- *Bone volume fraction (BV/TV):* increases with ZOL and RAL+ZOL in both WT and Het
- *Trabecular thickness*: increases with RAL and RAL+ZOL in WT, only RAL in Het
- *Trabecular number:* increases with ZOL and RAL+ZOL in both WT and Het
- *Bone mineral density (BMD):* increases with ZOL and RAL+ZOL in both WT and Het
- *Tissue mineral density (TMD):* increases with RAL and RAL+ZOL in WT, only RAL+ZOL in Het

Cortical Properties

- Only significant increase in WT cortical thickness with RAL+ZOL
- No significant changes to cortical TMD, BMD, moment of inertia, or cross sectional area

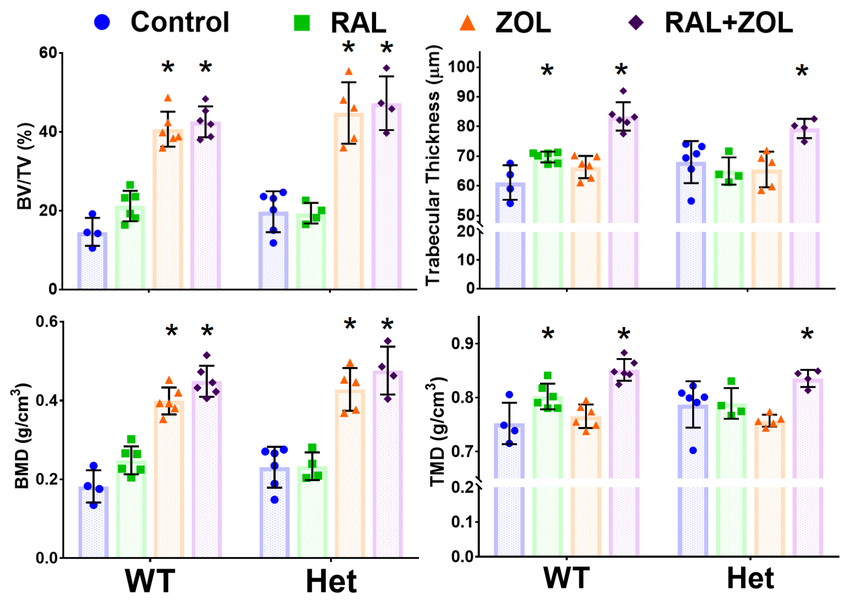

Figure 2. Trabecular architecture in distal femur for BV/TV, trabecular thickness, BMD, and TMD as bar (mean) and whiskers (standard deviation). *Indicates p<0.05 vs. control group within genotype

Mechanical Testing

- *Ultimate stress:* significant increases with RAL+ZOL in WT; RAL and RAL+ZOL in Het
- Nonsignificant increases seen in yield stress, failure stress, toughness, and total work

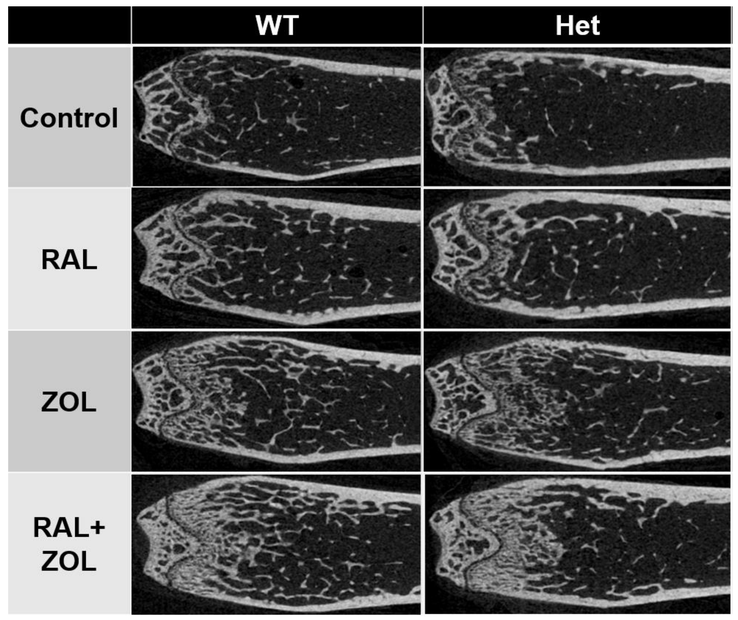

Figure 3. Distal femur CT images of each treatment for each genotype.

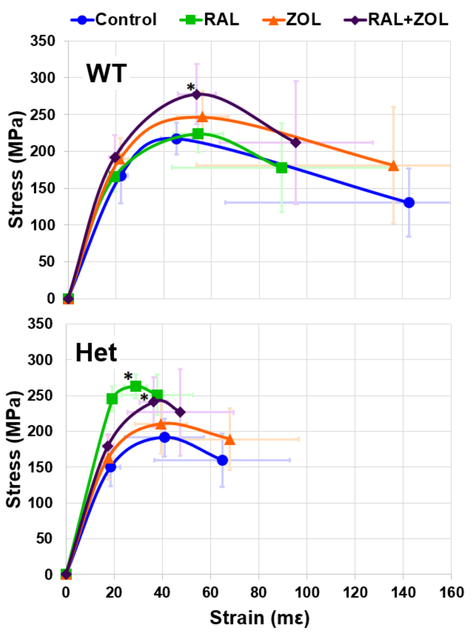

Figure 4. Stress-strain curve for WT and Het samples. *Indicates p<0.05 in ultimate stress vs. control group within genotype

CONCLUSIONS

Combination treatment of RAL and ZOL showed the most significant increases in trabecular architecture, with promising enhancement of mechanical properties.

The heterogeneous nature of the diseased bone most likely led to the variability in mechanical properties.

Future work includes increasing sample size and studying more therapeutic combinations to optimize bone quality and quantity.

References: [1] Gallant MA, et al. Bone. 2014;61:191-200; [2] Rao SH, et al. J Biomech. 2008 Dec 5; 41(16): 3371–3376.