Zoledronate and Raloxifene Combination Therapy Enhances Material Properties of Mouse Bone

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INTRODUCTION

Skeletal Fragility

- Estimated 1.5 million fractures and $18 billion in United States every year[1]
- Caused by numerous bone diseases and disorders
- One example: “OI” Osteogenesis Imperfecta (genetic cause)
  - Also known as “brittle bone” disease
  - Caused by mutation in Type I collagen or related proteins

Raloxifene (RAL)

- FDA-approved agent to treat osteoporosis in postmenopausal women
- Mechanism #1: cell-dependent as Selective Estrogen Receptor Modulator (SERM)
- Mechanism #2: cell-independent by binding to the collagen/mineral interface and increasing tissue hydration[2]

Zoledronate (ZOL)

- Common bisphosphonate (BP) used to treat osteoporosis and other bone disorders (OI)
- Inhibits resorption by osteoclasts, leading to increases in bone mass and BMD
- Mass-based effects are seen with BPs, however, bone quality is not necessarily improved[3]

HYPOTHESIS

Using the mass-based effects of ZOL, in conjunction with the quality-based changes of RAL, will enhance mechanical and materials properties of the bone more than either treatment alone.

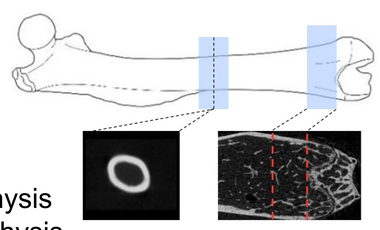
MATERIALS AND METHODS

Animals and Treatment

- Heterozygous (OIM+/-) and Wild-type (WT) male mice from OI murine model
- Treatment began at 8 weeks; mice euthanized at 16 weeks
- SubQ injections of RAL (0.5 mg/kg; 5x/wk), ZOL (80 µg/kg; once at 8 wks and 12 wks), or both
- n = 13-15 per group

Microcomputed Tomography (µCT)

- Right femora scanned at 10-micron resolution
- 1mm ROI at distal metaphysis and mid-diaphysis

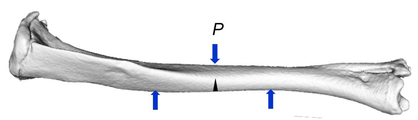


Mechanical Testing

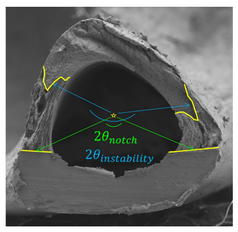
- Femora tested to failure in 3-point bending (anterior surface in tension)

Fracture Toughness (Tissue Quality)

- Right tibiae notched on anteromedial surface
- Tested to failure in 3-point bending (notched surface in tension)



- Graded EtOH dehydration (70-100%)
- SEM to determine angles of stable and unstable crack growth
- Analysis of toughness at crack initiation, maximum load, and crack instability



Statistical Analysis

- One Way ANOVA with Post-hoc Dunnett’s tests to analyze effects of each treatment versus control within each genotype

RESULTS

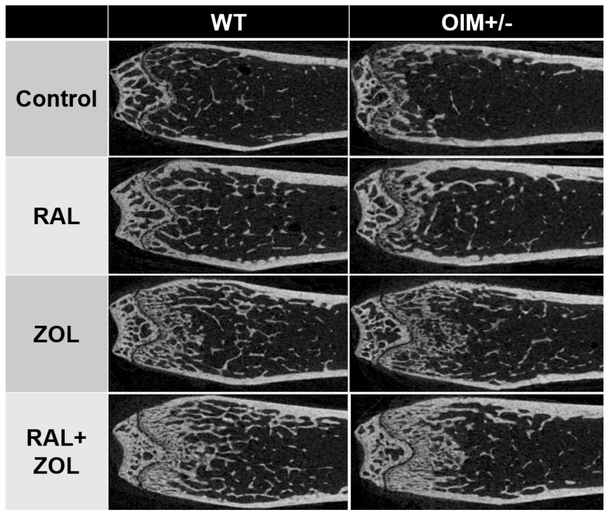


Figure 1. Sample of trabecular architecture in distal femur for each group.

Table 1. Percent change from control within each genotype. Green indicates significant change.

Property	WT			OIM+/-		
	RAL	ZOL	RAL+ZOL	RAL	ZOL	RAL+ZOL
Bone Volume Fraction (%)	ns	+95.5%	+94.4%	ns	+130.9%	+132.0%
Trabecular Thickness (µm)	+7.1% (p=0.07)	ns	+24.7%	+5.8% (p=0.09)	ns	+20.9%
Trabecular Separation (mm)	ns	-40.2%	-28.2%	ns	-50.6%	-38.6%
Trabecular Number (1/mm)	ns	+97.9%	+56.9%	ns	+137.9%	+94.4%
Bone Mineral Density (g/cm^3)	ns	+69.8%	+84.7%	ns	+90.4%	+108.1%
Tissue Mineral Density (g/cm^3)	+4.8%	ns	+11.0%	ns	ns	+8.7%

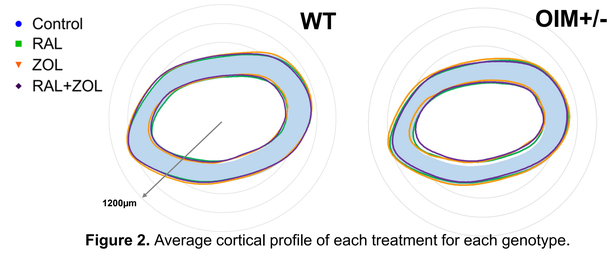


Figure 2. Average cortical profile of each treatment for each genotype.

Bone Microarchitecture – *Figure 1 & 2*

- Trabecular increases driven by ZOL (BV/TV, BMD, number, separation) – *Table 1*
- Increases in trabecular thickness and TMD only with combination treatment
- No significant changes in any cortical geometric parameters

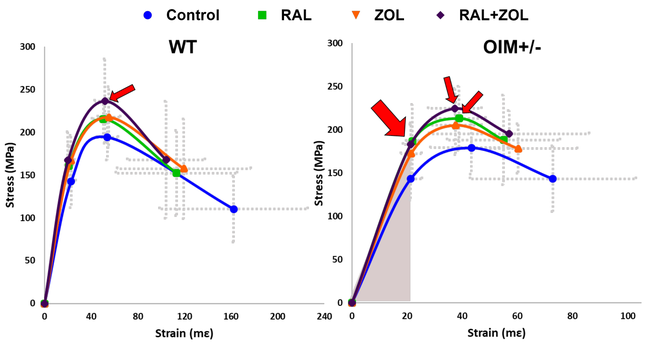


Figure 3. Schematic stress-strain curve of average estimated tissue-level properties for each treatment.

Fracture Toughness – *Figure 4*

- Significant increase in toughness at crack initiation and maximum load in OIM+/- with combination treatment
- No changes with either monotherapy
- No changes with any treatment in WT

Mechanical Properties – *Figure 3*

- Yield stress increased with all treatments in OIM+/-, no significant elevation in WT
- Ultimate stress increased with combo and RAL in OIM+/-, only significant increase in WT was with combo
- Resilience increased with combo and RAL in OIM+/-

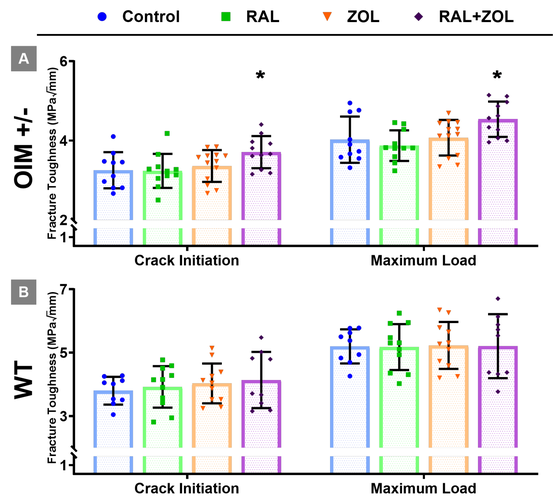


Figure 4. Average fracture toughness at crack initiation and maximum load within each genotype. Significant change from control indicated by ‘*’ at p<0.05.

CONCLUSIONS

Combination of RAL and ZOL treatment enhanced trabecular architecture, mechanical properties, and fracture toughness in diseased mouse bone

Future work includes investigating combination treatment in older animals, both sexes, different dosing schedule, and different drugs

References: [1] Reports of the Surgeon General. 2004; [2] Gallant MA, et al. Bone. 2014;61:191-200; [3] Rao SH, et al. J Biomech. 2008 Dec 5; 41(16): 3371–3376.