Exploring the use of novel raloxifene analogs as extracellular bone therapeutics in the *Col1a2*$^{G610C/+}$ murine model of osteogenesis imperfecta

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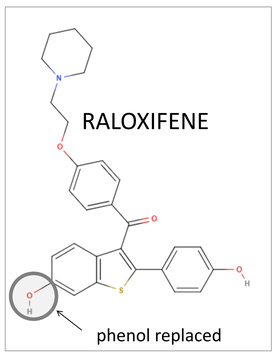
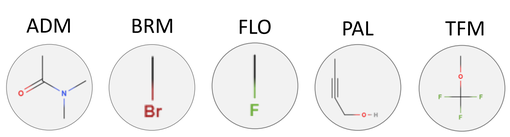
INTRODUCTION

- Osteogenesis imperfecta (OI) is a class of genetic disorders with altered collagen formation that can cause severe skeletal deformity and increased fracture risk.
- Current treatments have limited efficacy as bone-mass targeting drugs (e.g. bisphosphonates) do not address underlying tissue-level weakness in OI.
- Raloxifene (RAL), a selective estrogen receptor modulator (SERM), has been shown to improve bone quality by increasing tissue hydration, however RAL cannot be administered to pediatric patients due to its hormonal activity.
- Our lab previously showcased a RAL analog with reduced estrogen (ER) binding affinity but maintained ability to increase the toughness of OI bone. Here we attempted to build and improve upon that previous work:

AIM: Find a RAL analog with little-to-no ER activity but maintained ability to improve OI bone quality.

ANALOG STRUCTURE and ANALYSIS





ANALOG STRUCTURE and SYNTHESIS

- 5 new RAL analogs were designed and tested
- Synthesized and purified by flash column chromatography and crystallization
- In each analog the C-6 phenol of RAL (left) was replaced with an alternative group that could retain hydrogen bonding characteristics (above).

ANALOG ANALYSIS

Analogs were tested for ER binding affinity and impact on cell (MC3T3 murine pre-osteoblasts) viability and *C3* gene expression in comparison to 17β-estradiol (17βE).

RAL-ADM was chosen as the best candidate for in-vivo study.

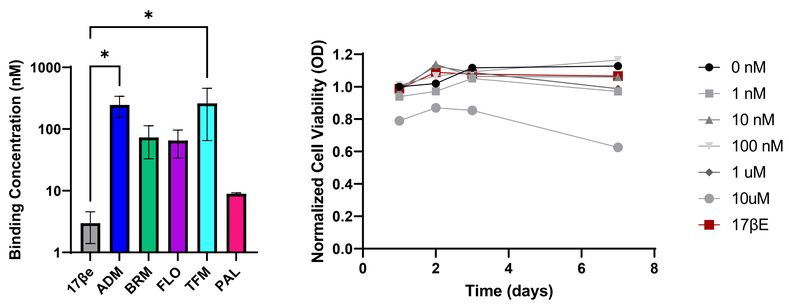


Figure 1. RAL-ADM has little estrogenic activity and impact on cell viability. (Left) Average IC50 binding concentration values from fluorescence polarization assays show ADM and TFM have reduced ER binding affinity compared to 17βE. (Right) MTT tests for cell viability over a week of growth showed consistently low rates of cell death up to a media concentration of 10 uM for RAL-ADM, similar to 17βE.

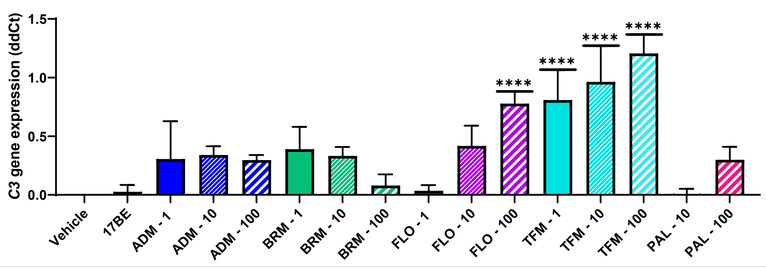


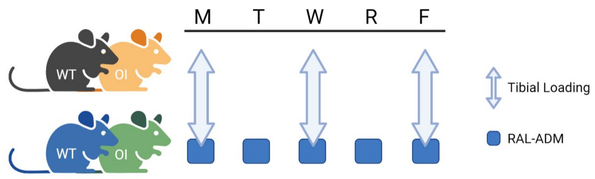
Figure 2. **RAL-ADM shows reduced ER signaling.** qPCR showed reduced *C3* expression (a downstream product of ER signaling), in RAL-ADM treated cells compared to other analogs.

IN-VIVO WORK

METHODS

Study Design

- Female C57BL/6 (WT) and *Col1a2*^{G610C} (OI) mice were randomly assigned to treated or untreated groups (n=15): **WT-Control**, **WT-ADM**, **OI-Control**, and **OI-ADM**.



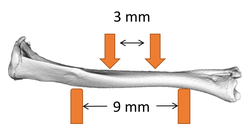
- 10-week-old mice underwent compressive tibial loading 3×/week (to 2050 με of tension) and RAL-ADM treatment (0.5 mg/kg; 5×/week) for 6 weeks, then sacced. (See figure above.)
- RAL-ADM was prepared in a 10% hydroxyl-β-cyclodextrin solution and injected subcutaneously.

Micro-Computed Tomography

- Right tibiae (RT) and hydroxyapatite phantoms were scanned via μCT (10 μm resolution) with a Bruker Skyscan 1172
- Scans were analyzed for cortical and trabecular properties using CTAn and MATLAB

Mechanical Analysis

- RT were tested to failure in 4-point bending (right), at a displacement rate of 0.025 mm/s.
- μCT scans of failure sites were used to calculate mechanical properties.



Statistical Analysis

- Differences between groups were assessed statistically using 2-way repeated measure ANOVAs, with WT and G610C groups being considered separately. (Main effects: loading, treatment)

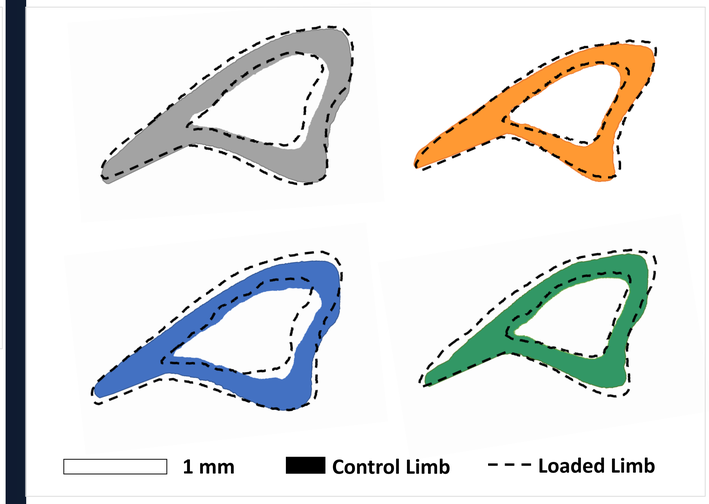


Figure 3. Only loading increased bone mass. Cortical profiles from one representative specimen from each group, showing how WT mice had a greater loading response than OI, with RAL-ADM not affecting bone mass.

RESULTS

- *In-vivo* treatment of RAL-ADM with and without loading resulted in little change to trabecular or cortical bone in G610C animals (Figs 3, 4)
- RAL-ADM had no effect on whole bone or tissue-level strength (Fig 5).

DISCUSSION

- Little change in cortical and trabecular bone mass suggests reduced estrogenic activity
- However, RAL-ADM did not retain the ability to improve bone strength in OI animals
- The focus on removing ER binding, while important, may not be a sufficient segregator of analogs, as hydration effects must also be measured.
- The muted response to loading in G610C mice demonstrates that OI may also reduce bone's mechano-sensitivity, a finding that has been observed and requires further investigation

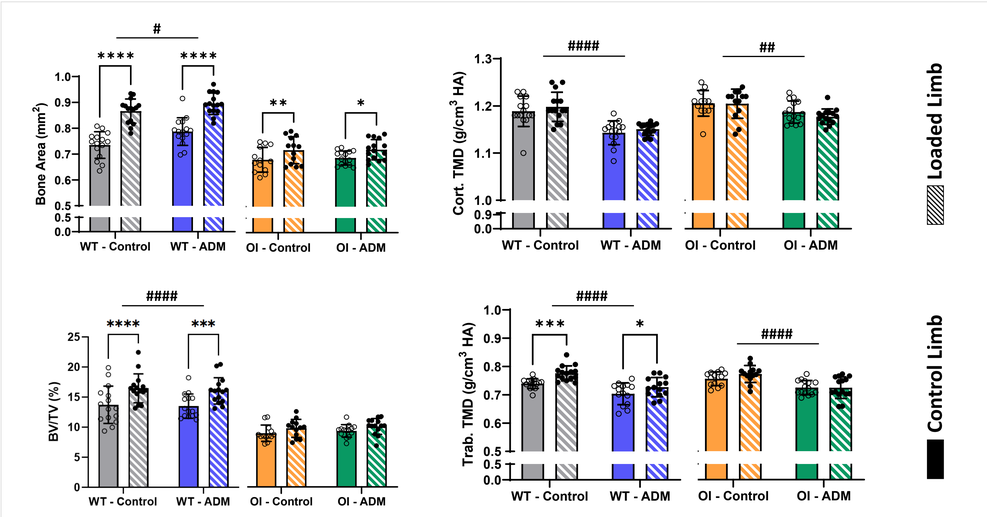


Figure 4. RAL-ADM had little impact on bone remodeling in OI. In both WT and OI mice, loading (*) had the most impact on cortical (top) and trabecular (bottom) bone mass, with RAL-ADM (#) lowering TMD.

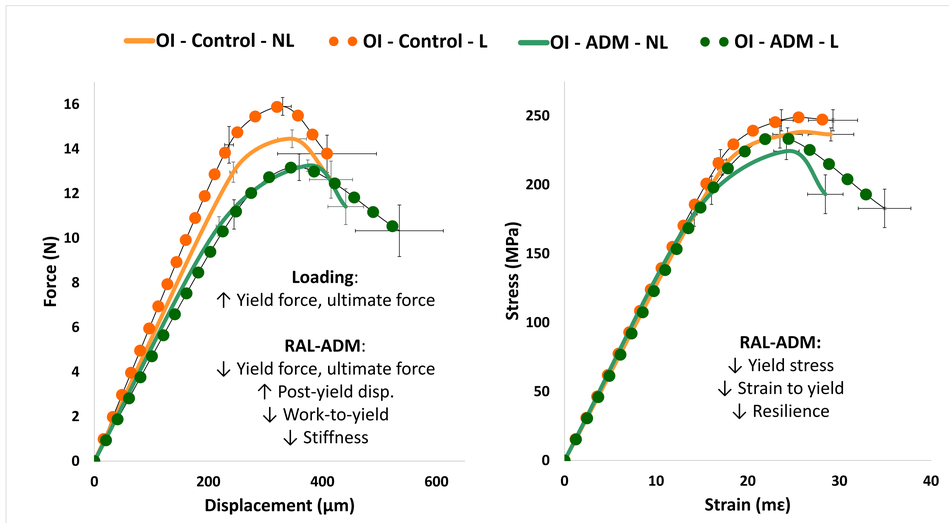


Figure 5. RAL-ADM induced no improvement in bone strength. Average force-displacement (left) and stress-strain plots (right) show that RAL-ADM treatment had little-to-no positive impact on whole-bone or tissue-level mechanical properties in OI mice. NL = Control Limb, L = Loaded Limb, bars show standard error.

CONCLUSION: Estrogenic affinity of RAL-ADM was successfully reduced but did not produce an improvement in OI bone quality.

ACKNOWLEDGEMENTS

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