Combining Targeted Tibial Loading and Raloxifene Improves Bone Structure and Quality in Female Mice from the G610C Model of Osteogenesis Imperfecta

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Introduction

Osteogenesis imperfecta (OI) is a condition of low bone mass and poor bone quality due to mutations of collagen or its processing proteins. [1]

Current therapies improve bone quantity but not the quality of new bone. [2]

Compressive tibial loading can improve bone structure in mice. [3]

Raloxifene has reduced spontaneous fractures in preclinical models of severe OI by improving bone quality. [4]

We hypothesize that using a combination of targeted tibial loading and raloxifene treatment will improve the quantity and quality of bone in an OI mouse model.

Study Design

Animals

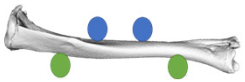
- G610C +/- (OI) and WT female mice were given raloxifene (RAL) injections (0.5 mg/kg) 5 days per week and subjected to tibial loading for 3 days per week starting at 10 weeks of age and continuing for 6 weeks

Tibial compression
Main et al. Journal of Orthopaedic Research. 2020.

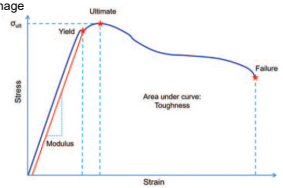
µCT/Mechanics

- Tibiae scanned at 9.8 µm voxel
- Broken in 4-point, displacement rate of 0.025 mm/sec
- Group comparisons were performed for each genotype using RM 2-way ANOVAs to determine effects of loading and raloxifene

µCT Image



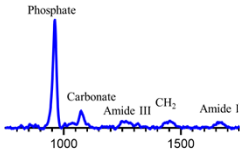
4-pt Bend Test Setup



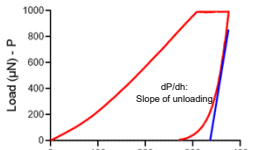
Stress Strain Curve

Raman/nanoindentation

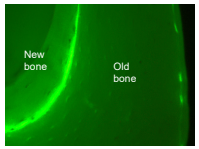
- Transverse cross-sections were cut from embedded tibiae at 37.5% length from the proximal end
- Colocalized Raman (785 nm laser) and nanoindentation (1.03 µm radius spherical probe) were performed on new bone formed during loading (new) and bone formed prior to loading (old) as determined by calcein labels
- RM 3-way ANOVAs used to determine effects of genotype, age of bone, and raloxifene treatment



Raman spectra

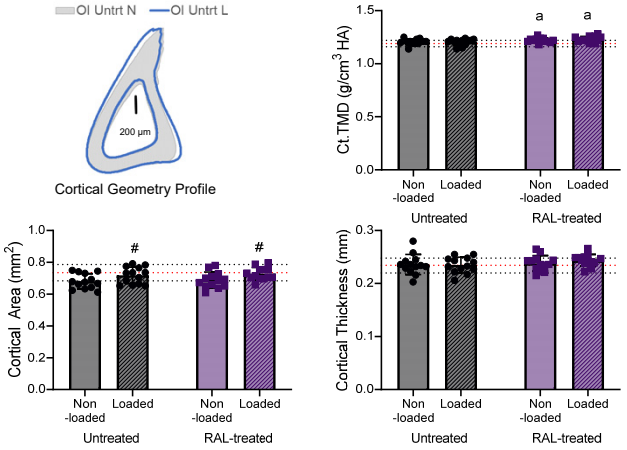


Nanoindentation Curve

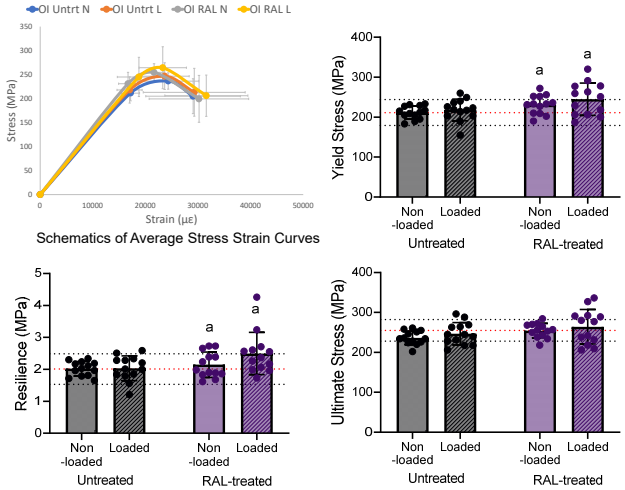


Calcein Labels for Determining Bone Age

Loading Improved Bone Structure and Raloxifene Improved Bone Material Properties in OI Mice



Cortical tissue mineralization density (Ct.TMD) was higher with RAL treatment as indicated by a. Cortical area was higher in loaded bone as indicated by #. Thickness was not altered. Averages and standard deviations for WT non-loaded, untreated bone indicated by dashed lines.



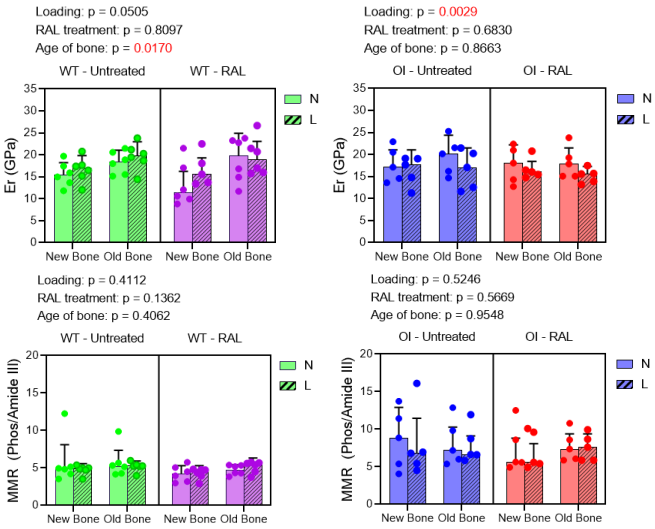
Tissue level material properties were altered by RAL treatment, but not loading as indicated by a. Averages and standard deviations for WT non-loaded, untreated bone indicated by dashed lines.

	WT				OI			
	% Change		Effect Size		% Change		Effect Size	
Property	L	L+R	L	L+R	L	L+R	L	L+R
Cortical area	17.9	20.9	2.67	2.94	5.44	6.89	0.74	1.07
Cortical thickness	9.89	13.2	1.78	2.02	-0.48	3.16	0.07	0.48
Ct.TMD	0.78	0.10	0.29	0.04	-0.12	2.10	0.05	0.92
Yield stress	7.79	10.6	0.47	0.57	3.08	15.8	0.29	1.08
Ultimate stress	1.21	3.51	0.11	0.31	4.32	11.7	0.44	0.84
Resilience	17.0	21.4	0.72	0.73	0.64	23.7	0.04	0.97

L = Loading, L+R = Loading, RAL-treatment. All comparisons made to untreated, non-loaded bones

Percent change (% Change) and Cohen's d effect size determined for loaded untreated bone and loaded RAL-treated bone as compared to non-loaded untreated bone. % Change and Effect Size are larger for WT females for structural properties, and larger for OI mice for material properties

Loading Altered Nanoscale Mechanical Properties



Modulus (Er) was not altered with RAL treatment but was altered with loading. Er was higher for loaded WT mice and was lower for loaded OI mice. Er was only changed with age of bone in WT mice, with old bone having a higher Er than new bone. Mineral to matrix ratio (MMR) was not changed with either loading, treatment, or age of bone. Three way ANOVA p values above graphs, no significant interactions were observed.

Discussion

OI mice are able to build bone in response to loading, though this response is less robust than WT mice

Raloxifene improved material properties (yield stress and resilience) in OI mice. While there were no significant improvements in post-yield properties, these did not worsen with the increased mineralization.

Modulus changes were different based on genotype, which may have been the result of initial mineralization values

Loading altered nanoscale material properties, while raloxifene improved whole bone material properties..

Combining loading and raloxifene treatment improves bone structure and quality. Further investigation will be needed to determine the mechanism of improved quality.

References

[1]Forlino. 2016. Lancet. 387(10028): 1657–1671 [2]Morello. 2018. Matrix Biol. 71-72: 294-312 [3] Main. 2019, J Orthop Res. 38: 233-253. [4] Berman. 2016. Matrix Biol. 52-54: 19-28.

Lab QR Code

Acknowledgments

This work was funded by grants AR065971 and AR072609 from the NIH.