Raloxifene Reduces Skeletal Fractures in Homozygous OIM Male Mice

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INTRODUCTION

Osteogenesis Imperfecta (OI)

- Heritable bone disease caused by mutation in genes encoding Type I collagen
- Characterized by small, weak bones that have low energy to fracture
- Difficult to treat due to impacts on bone throughout its hierarchy
- Current treatments improve fracture incidence, but do not recover properties

Raloxifene (RAL)

- FDA-approved pharmacological agent
- Clinically used to decrease fracture incidence in post-menopausal women
- Recent work has shown that RAL improves quantity AND quality
  - Increases bound water in bone
  - Leads to improved post-yield mechanical properties

HYPOTHESIS

Raloxifene will produce beneficial effects on bone mechanical properties in a mouse model of OI

MATERIALS AND METHODS

Experiment Design #1: Ex vivo effects of Raloxifene

Animals and Sample Preparation

- Paired tibiae from 12 week old homozygous OIM (B6C3Fe a/a-Col1a2oim/Col1a2oim) and WT (B6C3FeF1/J a/a) female mice
- Soaked in either PBS (left tibiae) or 2 μM raloxifene (right tibiae) for 13 days
  - Incubated at 37° C
  - Supplemented with 1% Pen/Strep

Mechanical Testing

- Four-point bending to failure
  - 9 mm bottom support; 3 mm loading span
- Displacement rate of 0.025 mm/sec
- Tested in the medial-lateral direction with the medial side in tension

Experiment Design #2: In vivo effects of Raloxifene

Animals

- OIM and WT male mice (8 weeks)
- Injected 5 days/week for 8weeks with saline vehicle or 0.5 mg/kg raloxifene
- At 16 weeks of age, mice euthanized

Dual-energy X-ray Absorptiometry (DXA)

- Mice scanned immediately post-mortem
- Regions of interest (ROI): whole body, femur, and L4-L5 vertebrae

Microcomputed Tomography (CT): Femurs

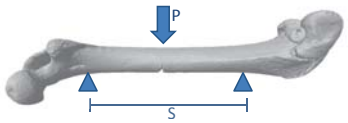
- 9.8 μm resolution
- Fracture Assessment
  - Projection images used to score femurs as intact or fractured
- Cancellous Analysis
  - Distal metaphysis (10% of bone length)
- Cortical Analysis
  - ROI: 40% of the bone length from distal

Mechanical Testing: Tibiae

- Prior to testing, CT at 16.8 μm resolution
- Four-point bending, same as above
- CT slices at the location of fracture were used to convert Force-Displacement into Stress-Strain

Fracture Toughness Testing: Femurs

- Notched on anterior surface
- 3 point bending at 0.001 mm/sec
- SEM to determine angles of stable and unstable crack growth
- CT at fracture site to determine geometry



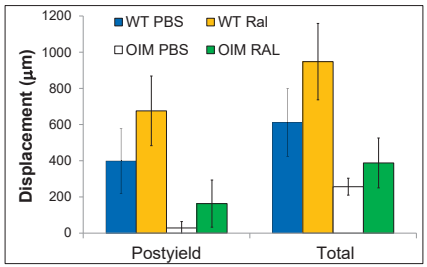
Statistical Analysis

- Ex vivo study #1
  - Repeated measures ANOVA
  - If interactions, pairwise t-tests with Bonferroni correction ($p<0.0125$)
- In vivo study #2
  - Two-Way ANOVA with the date of arrival was blocked as a nuisance factor
  - Fisher's Exact Test for fracture assessment of OI VEH versus RAL

RESULTS AND DISCUSSION

Experiment Design #1: Ex vivo effects of Raloxifene

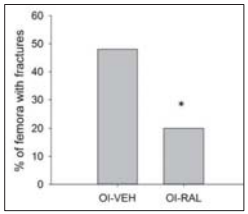
- Investigates non-cellular effects of RAL
- OIM resulted in:
  - ↓ Force (yield and ultimate)
  - ↓ Displacement (yield, post-yield and total)
  - ↓ Stiffness
  - ↓ Work (yield, post-yield, and total)
- Treatment with raloxifene resulted in:
  - ↑ Post-yield and total displacement
  - ↑ Work to failure

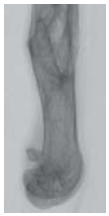


Experiment Design #2: In vivo effects of Raloxifene

Fracture Assessment

- # of fractures ↓ with raloxifene treatment





Fracture Toughness

- OI bones had significantly lower resistance to crack propagation
- Raloxifene treatment improved stress intensity factor at maximum load

Mechanical Testing to Failure

- OI bones had significantly lower mechanical properties
- No significant effects of raloxifene treatment

Cancellous Architecture

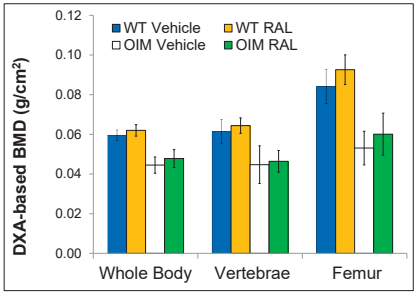
- OIM resulted in:
  - ↓ Bone volume fraction (BV/TV)
  - ↓Trabecular number and thickness
  - ↑ Trabecular separation
  - ↓ BMD
- Treatment with raloxifene resulted in:
  - ↑ Bone volume per tissue volume
  - ↑ Trabecular thickness

Cortical Architecture

- OIM resulted in less cortical bone
- Raloxifene treatment ↑ cortical thickness

Dual-energy X-ray Absorptiometry

- BMD ↑ in whole body and femur with RAL



CONCLUSION

- **In accord with previous data, Osteogenesis Imperfecta is characterized by smaller, weaker bones with decreased mechanical integrity**
  - Results indicated diminished cortical and cancellous architecture in conjunction with decreased mechanical properties (both pre- and post-yield)
- **Raloxifene treatment resulted in decreased fracture incidence**
  - Improvements to mechanical integrity by traditional testing techniques were minimal
  - Raloxifene-treated mice had significantly fewer fractures

Raloxifene significantly reduced the number of fractures in OI mice, providing initial framework for an alternative approach to treating OI.