The rat model of osteoporosis

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Outline of Presentation

• Osteoporosis definition and impact
• Characteristics of the ovariectomized rat model
• Methods of evaluating osteopenia
• Publications of the effects and mechanisms of substances
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• Osteoporosis definition and impact

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Definition of osteoporosis

- Osteoporosis is a metabolic disease characterized by loss of bone mass and deterioration of bone microarchitecture, which leads to increased fragility and can result in spontaneous or low energy fractures.

Definition of osteoporosis

- Osteoporosis: bone density has decreased more than -2.5 SD (T-score) of the mean bone density of healthy young adults.
- Osteopenia: bone density < -2.5 SD
- Osteopenia: -2.5 SD < bone density < -1 SD
The “silent disease”

- Gradual loss of bone mass is not accompanied by any symptoms
- Its victims may pass the fracture threshold unawares
- Spontaneous or low energy fractures may occur during coughing, sneezing, bending, or rolling over in bed
- 35% of women > 65 years suffer from osteoporosis
Osteoporosis-related fracture sites

- Osteoporotic fractures usually occur in areas of bone significantly affected by bone loss, such as the hip, the forearm and the spine.
- Hip fractures in particular present major impacts to a person’s health, quality of life and health care costs.
Impact of osteoporosis

- Health impact:
  Mortality 10% – 20% in the 6 months following a hip fracture

- Socioeconomic impact:
  The cost of osteoporotic fractures, including hospitalization and rehabilitation, is enormous and tends to increase.

Impact of osteoporosis

Europe total osteoporosis costs: 36.3 billion euros in 2000, projected costs: 76.8 billion euros in 2050.

J.A. Kanis, WHO Collaborating Centre for Metabolic Bone Diseases, 2005
Limitations of clinical studies

- Disease appears at advanced ages
- Many years required for follow-up
- Many patients drop out
- Many patients do not adhere to treatments
- Patients have diverse backgrounds (lifestyle, co-morbidities, other concurrent drug therapies, …)
Advantages of animal studies

- Animals’ life cycle is shorter, so the condition can be reproduced and studied faster
- Allow follow-up of the same animals (own controls) and contribute to Reduction
- Allow efficacy and safety testing of potential therapies
- Variability is minimized with animals of the same genetic background and in a controlled environment
Animal species used

- Non-human primates
- Dogs
- Rats
- Mice
- Rabbits
- Guinea pigs
- Mini-pigs
- Sheep
Rats

- Estrus cycles every 5 days
- Well characterized skeleton
- Short life-span
- Readily available
- Inexpensive
- Ease of maintenance
- Accepted by society

Fig. 2. A compilation of publications using animals as models for osteoporosis between 1966 and 1998. The number of publications is shown by year for rats, mice, dogs and monkeys.

The rat model of osteoporosis research

Hormonal interventions

Dietary interventions

Immobilization

Surgical

Pharmaceutical

Low calcium diet

Conservative

Surgical

Alcohol abuse

Other

Nerve resection

Gonadectomy

Low calcium diet

Nerve resection

Hypophysectomy

Alcohol abuse

Tendon resection

Parathyroidectomy

Other

Spinal cord resection

Gonadectomy

GnRH agonists

Limb bandaging

Ovarectomy

Estrogen receptor antagonists

Limb casting

Gonadectomy

Aromatase inhibitors

Tail suspension

Gonadectomy

Hypothyroidism

Surgical

Hypophysectomy

Pharmaceutical

Surgical

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Characteristics of the Ovx rat

- Increased bone turnover after ovariectomy (Ovx): bone resorption > bone formation
- Initial rapid phase of bone loss followed by a slower phase of bone loss
- Trabecular bone loss > cortical bone loss
- Bone response to therapy with estrogen, SERMs, bisphosphonates, parathyroid hormone and exercise
- Possibility of measuring bone loss
- Osteopenia does not lead to fragility fractures
Characteristics of the Ovx rat

- **Peak bone mass is acquired at 9 ms age**
- **Ovx before 9 ms age**: trabecular bone loss, due to altered bone growth
- Appropriate model for endocrine, nutritional & environmental factors on peak bone mass
- **Ovx after 9 ms age**: trabecular and cortical bone loss, due to altered bone remodeling
- Appropriate model for postmenopausal osteoporosis
- Site- and time-specific bone loss

Site- / time-specific bone changes

<table>
<thead>
<tr>
<th>Site</th>
<th>LBG** at 9 months</th>
<th>Earliest time of bone loss</th>
<th>Time of 50% bone loss</th>
<th>Earliest to achieve steady state</th>
<th>Earliest decrease in bone strength</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTM</td>
<td>3 μm/d</td>
<td>≈ 14d</td>
<td>≈ 30-60d</td>
<td>90d</td>
<td>--</td>
<td>18,19</td>
</tr>
<tr>
<td>LVB</td>
<td>&lt; 1 μm/d</td>
<td>≈ 60d</td>
<td>≈ 180-270d</td>
<td>270d</td>
<td>90d</td>
<td>20(43,45)</td>
</tr>
<tr>
<td>FN</td>
<td>&lt; 1 μm/d</td>
<td>≈ 30d</td>
<td>≈ 180-270d</td>
<td>270d</td>
<td>90d</td>
<td>14(39,44)</td>
</tr>
<tr>
<td>DTM</td>
<td>closed</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>--</td>
<td>26</td>
</tr>
</tbody>
</table>

The times (d) listed may be less than that listed due to lack of short term studies; **LBG – longitudinal bone growth, PTM – proximal tibial metaphysis, LVB – lumbar vertebral body, FN – femoral neck, DTM – distal tibial metaphyses, -- no determination, ( ) bone strength references.

Table 1. Summary of cancellous bone changes post-ovariectomy.

The Ovx rat
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Methods of evaluating osteopenia

Non-invasive methods

- Bone densitometry:
  - Dual Energy X-ray Absorptiometry (DEXA),
  - peripheral Quantitative Computed Tomography (pQCT),
  - micro CT (μCT)
- MRI
- Biochemical markers of bone turnover

Invasive methods

- Bone histomorphometry
- Bone mechanical strength tests
DEXA measurement of BMD

• Brief injectable anesthesia (ketamine hydrochloride and dexmedetomidine, reversal with atipamezole)
• Correct positioning
• Small animal software scans of bone mineral density (BMD) pre-Ovx, intra-therapy and post-therapy
DEXA measurement of BMD

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Total and proximal tibia ROIs
DEXA measurement of BMD

Mean percentage change of Proximal Tibia from baseline (%) vs Time (months)

Control
OVX
OVX+G

a : p<0.0005 vs OVX

0 3 6

Time (months)
DEXA measurement of BMD
DEXA measurement of BMD

1. Mean percentage change from the baseline of bone mineral density (BMD) of the proximal tibia during the treatment period.

2. Mean percentage change from the baseline of bone mineral density (BMD) of the total tibia during the treatment period.
pQCT

- pQCT evaluates trabecular bone separately from cortical bone
- Slices of the tibia at 3, 4 and 14 mm distal to the knee joint, pre-Ovx, intra- and post-therapy
MRI

- MRI image of rat trabecular bone

Invasive assessment: Histomorphometry

- Evaluates bone architecture and fragility independently of bone mass
- 2-dimensional analysis, provides static parameters (number of osteoblasts, osteoclasts, trabeculae, trabecular thickness & separation) and dynamic parameters (bone formation, mineral apposition rate)
Table 3
Comparison of histomorphometric variables among groups

<table>
<thead>
<tr>
<th>Parameter (value)</th>
<th>Group</th>
<th>Mean ± S.D.</th>
<th>Overall significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV/TV (%)*</td>
<td>Ovx</td>
<td>9.32 ± 2.15</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td></td>
<td>Ovx + Ph</td>
<td>13.00 ± 5.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25.86 ± 5.85</td>
<td></td>
</tr>
<tr>
<td>NOb/BPm (μm⁻¹)***</td>
<td>Ovx</td>
<td>11.170 ± 3.09</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Ovx + Ph</td>
<td>6.210 ± 4.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.383 ± 1.85</td>
<td></td>
</tr>
<tr>
<td>TbTh (μm)**</td>
<td>Ovx</td>
<td>46.92 ± 6.66</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Ovx + Ph</td>
<td>49.99 ± 7.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>57.20 ± 9.12</td>
<td></td>
</tr>
<tr>
<td>TbSp (μm)*</td>
<td>Ovx</td>
<td>469.74 ± 86.96</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td></td>
<td>Ovx + Ph</td>
<td>383.43 ± 137.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>167.59 ± 27.57</td>
<td></td>
</tr>
<tr>
<td>Tbn (mm⁻¹)*</td>
<td>Ovx</td>
<td>1.99 ± 0.37</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td></td>
<td>Ovx + Ph</td>
<td>2.53 ± 0.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.49 ± 0.49</td>
<td></td>
</tr>
</tbody>
</table>

Groups: ovariectomy (Ovx), ovariectomy plus plant extract (Ovx + Ph), sham-operated (control). See text for histomorphometry abbreviations.

* All pairwise comparisons between groups are statistically significant $p < 0.0005$, except Ovx + Ph vs. Ovx.

** All pairwise comparisons between groups are statistically significant $p < 0.05$, except Ovx + Ph vs. Ovx.

*** There is statistically significant difference between Ovx vs. Ovx + Ph $p < 0.05$. 
Ex vivo bone strength

- Three- or four-point-bending of the tibia or femur
- Cantilever testing of the femoral head
- Compression testing of vertebrae
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Publications with the Ovx rat model

1. **TRAF6 mediates suppression of osteoclastogenesis and prevention of ovariectomy-induced bone loss by a novel prenyllavonoid.**
   - Tan EM, Li L, Indran IR, Chew N, Yong EL.
   - PMID: 27813153

2. **Alendronate does not prevent long bone fragility in an inactive rat model.**
   - J Bone Miner Metab. 2016 Nov;34(6):615-626.
   - PMID: 26475371
   - Similar articles

3. **Aspirin prevents bone loss with little mechanical improvement in high-fat-fed ovariectomized rats.**
   - PMID: 27615444
   - Similar articles
Publications with the Ovx rat model

The Laboratory Rat as an Animal Model for Osteoporosis Research

Pavlos P. Lelovas,1 Theodoros T. Xanthos,2 Sofia E. Thoma,1 George P. Lyritis,1 and I mamene A. Don t as1,3

Available online at www.sciencedirect.com

Protective effect of plant extract from Onobrychis ebenoides on ovariectomy-induced bone loss in rats

I. Don t as1,4, M. Halabalaki b, P. Moutsatsou c, S. Mitakou b, Z. Papoutsi c, L. Khaldi a, A. Galanos b, G. P. Lyritis a

a Laboratory for the Research of the Musculoskeletal System, School of Medicine, University of Athens, Greece
b Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Greece
c Laboratory of Biological Chemistry, School of Medicine, University of Athens, Greece

Effects of high-intensity swimming training on the bones of ovariectomized rats

Tae woong Oh1, Sakura Tana la2#/# Tatsuki Naka1 # Shoji Igawa2


Bone mass improved effect of icariin for postmenopausal osteoporosis in ovariectomy-induced rats: a meta-analysis and systematic review.

Xu JH1, Yao M, Ye J, Wang QD, Wang J, Cui XJ, Mo W

I. Don t as
Conclusion

• The severity of osteoporosis in humans has led to the search of preventive and therapeutic methods tested on animal models, of which the laboratory rat has been most studied and has and is still yielding valuable results.
Thank you for your attention!