Which Model for Which Study
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Animal Models for Bone and Joint Diseases
Bone diseases
• Osteoporosis
• Bone metastasis (bone pain)

Joint diseases
• Rheumatoid arthritis
• Osteoarthritis

Other models
Gene deletions (knock-out), overexpression
Secondary osteoporosis – diabetes
- glucocorticoids, antiepileptica...
Senescence models
- C57BL/6, Senescence accelerated mice (SAM)
Fracture models
Mechanical loading

Why are animals used in biomedical research?
• organs and body systems similar to humans and other animals
• susceptible to the same diseases that affect humans
• short life span allows animals to be studied throughout their entire life
• environment easily controllable to keep experimental variables to a minimum

Animal model should mimic the human disease
- and one should understand the strengths and limitations of each model

Osteoporosis endpoints
Fractures
Surrogate endpoints
• Bone density
• Markers of bone turnover
• Bone structure
Clinical study with fluoride

Fluoride increases bone mass in vivo and osteoblast activity in vitro

4 year prospective clinical study comparing fluoride 75 mg per day and placebo showed a 10-35% increase in bone mineral density (Riggs BL. 1991 N Engl J Med. 322:802-9)

**Nonvertebral Fractures After 4 yrs of Fluoride Therapy**

<table>
<thead>
<tr>
<th>Location</th>
<th>Fluoride</th>
<th>Placebo</th>
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</thead>
<tbody>
<tr>
<td>Radius</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Humerus</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Rib</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Pelvis</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Proximal Femur</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Tibia</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Metatarsus or calcaneus</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>72</strong></td>
<td><strong>24</strong></td>
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**Comparison of Alendronate and NaF Effects on Bone Strength vs. Bone Mass**

![Graph showing comparison of Alendronate and Sodium Fluoride effects on bone strength vs. bone mass.](image)

**JCI, 95, 2127 (1995)**

**FDA guideline**

**GUIDELINES FOR PRECLINICAL AND CLINICAL EVALUATION OF AGENTS USED IN THE PREVENTION OR TREATMENT OF POSTMENOPAUSAL OSTEOPOROSIS**

April, 1994

**Animal models at different stages of drug development - osteoporosis**

Discovery (many compounds, small amount)
- Efficacy screening (short term/small animal)
- Mechanism of action

Development (one compound)
- Efficacy
- Safety
- Strength

**The TPTX Rat Model – for screening of anti-resorptive effect**

The Thyroid and Parathyroid glands are removed in male Sprague-Dawley rats.

This results in a low level of serum calcium.

After PTH infusion there is an increase of bone resorption.

This is measured as an increase in serum calcium.
The TPTX Rat Model – Treatment with Bisphosphonates

Serum calcium was measured at baseline and after 6 hours of PTH infusion.

Study designs for osteoporosis animal studies

Prevention or treatment?
Treatment regimen
Special models (glucocorticoid induced)
Animal species (rats, mice, monkeys...)
- strain
- age

Animal model of postmenopausal bone loss?

Menopause?
- ovariectomy
Bone remodeling
- Aged rats or mice or remodeling species
Fractures?
- Bone strength

Remodeling sites in rats

Values for femur mechanical properties determined by bend testing of 29 strains of mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pmax (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>13.7</td>
</tr>
<tr>
<td>129/J</td>
<td>23.1</td>
</tr>
</tbody>
</table>
Study Design
Animal model of osteoporosis

Ovariectomy (OVX)+/- treatment
Sham

Baseline 2 weeks 4 weeks 6 weeks
Postmortem tissue collection
Serum urine

Preclinical endpoints
- Bone mass/density:
  - ash weight, radiological methods (DEXA, pQCT, μCT)
- Biochemical markers of turnover
  - degradation and formation markers
- Histology, histomorphometry
  - OC and OB, bone volume, bone formation rate
- Biomechanical testing:
  - bending, torsion on long bones
  - compression of vertebrae

Measurements of Bone Density

Dual-Energy X-ray Absorptiometry (DXA or DEXA)

BMD (bone mineral density) g/cm²

Peripheral Quantitative Computed Tomography (pQCT)

BMD (bone mineral density) g/cm³

The Ovariectomized Rat Model

Trabecular Bone Mineral Density (mg/cm³)

Significantly different from vehicle: * p < 0.01

OVX-EE: 0.1
OVX-EE: 0.5
OVX-E4: 2.5
Section of rat tibia
bone volume/tissue volume (BV/TV)

Trabecular bone
Intact
Ovariectomized

Biomechanical Evaluations of bone strength

Compression test of femoral neck
Compression test of lumbar vertebral body
Three-point bending of femoral midshaft

Bone strength

Significantly different from vehicle: * p < 0.01

Markers of collagen degradation

Study Design
Animal model of osteoporosis

Ovariectomy (OVX)+/- treatment

Sham
Postmortem tissue collection

Density, strength, histomorphometry

Serum
Urine

Density, strength, histomorphometry

Weeks 2 4 6

Tetracycline label

Calcein label

Urinary Deoxypyridinoline/Creatinine
% Change from Baseline

 OVX
 Sham

Weeks after surgery

 OVX
 Sham

p= 0.03
Animal model of bone metastasis

Breast, prostate, lung, multiple myeloma

Syngeneic (mouse to mouse)

Orthotopic
Induced in breast or prostate tissue
Intracardiac, tail vein
Intrabone injection

Murine model of breast cancer-induced bone metastasis

Human breast cancer cells MDA-231

After 4 weeks

Arrows = Osteolytic metastasis

Effect of ibandronate in a mouse model of bone metastasis

Osteolysis

Vehicle ibandronate

Tracking tumour cells after intracardiac injection in mice

10 min 24 hours 28 days
Amount of osteolysis does not always correlate with tumor burden

Vehicle Ibandronate

Arthritis

Rheumatoid arthritis (RA)
- Chronic inflammatory disorder affecting multiple peripheral joints
- Autoimmune disease
- Synovial hyperplasia, immune cell infiltration, cartilage destruction, bone erosion

Rheumatoid arthritis models in rodents

Rat models
- Streptococcal cell wall (SCW)
- Antigen induced arthritis (AIA)
- Adjuvant arthritis (AA)
- Pristane-induced arthritis (PIA)

Mouse models
- Induced
  - Collagen-induced arthritis (CIA)
  - Pristane-induced arthritis
  - Proteoglycan-induced arthritis
  - Zymosan-induced arthritis
  - Immune complex arthritis
- Serum transfer models

Genetic


Arthritis

Osteoarthritis

Non-inflammatory degenerative joint disease, characterised by degeneration of the articular cartilage, hypertrophy of bone at the margins and changes in the synovial membrane.

Osteoarthritis animal models

- Physically induced
  - Meniscectomy
  - Anterior cruciate ligament transection
- Chemically induced
- Spontaneous
  - Hartley guinea pigs
  - Transgenic mice
  - Special mouse strains

Animal model of bone cancer pain
Cancer Pain

>70% of patients with advanced cancer suffer from cancer-related pain

the pain is most commonly related to bone metastasis (breast, prostate and lung cancer)

Bone cancer pain

Chronic pain
and
Breakthrough pain/transient pain

Treatment:
Opioids, NSAID, radiotherapy, bisphosphonates

Why does bone cancer hurt?

Increased pressure within bone?
Micro-fractures?
Compression of nerves?
Peripheral nerve damage?
Release of pro-nociceptive factors?
Acidic micro-environment?

Central sensitization in bone cancer pain

Spinal cord neurons are activated by stimuli that would normally be non-noxious

Different pain states?

Inflammatory pain
Neuropathic pain
Cancer pain?

The mouse model of bone cancer pain
Bone Destruction

\( \mu \text{CT-scanning} \)

Day 8  Day 7  Day 12

Day 10  Day 18  Day 22

Analysis of Pain-related behaviours

- Threshold for mechanical stimulation (von Frey)
- Limb use (open field and activity induced)
- Number of rearings
- Weight bearing (incapacitance test)
- Number of flinches
- Nocifensive behavior

Weight bearing

GFAP expression in the spinal cord

Sham  Cancer  WIN 55,212-2

\( n=14, 9 \text{ and } 8 \)

To consider when doing animal experiments:

Species  Strain  Age  Gender  Housing conditions  Chow, water