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

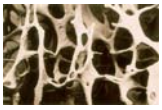
Why are animals used in biomedical research?

- 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*

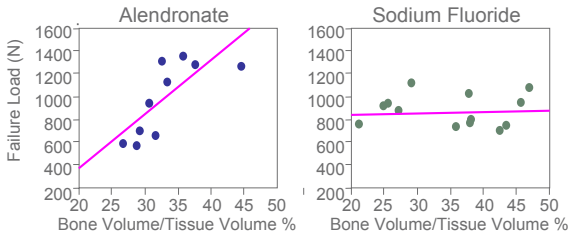
4 year prospective clinical study comparing fluoride 75 mg per day and placebo showed a 10-35% increase in bone mineral density (Riggs BL. 1991 N Engl J Med. 322:802-9)

Nonvertebral Fractures After 4 yrs of Fluoride Therapy

	FLUORIDE	PLACEBO
Radius	1	4
Humerus	6	1
Rib	14	8
Pelvis	7	1
Proximal Femur	13	4
Tibia	13	0
Metatarsus or calcaneus	12	3
Other	6	3
TOTAL	72	24

Riggs BL. 1991 N Engl J Med. 322:802-9.

Comparison of Alendronate and NaF Effects on Bone Strength vs. Bone Mass



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

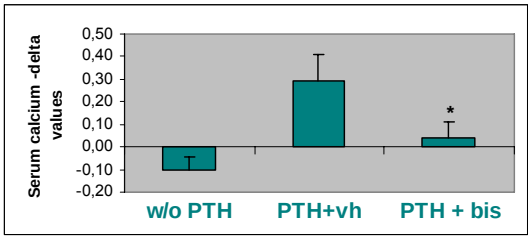
The Thyroid and Parathyroid glands are removed in male Sprague-Dawley rats.

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

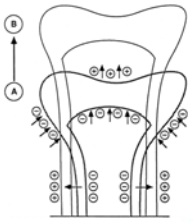
Animal model of postmenopausal bone loss?

Menopause?
- ovariectomy

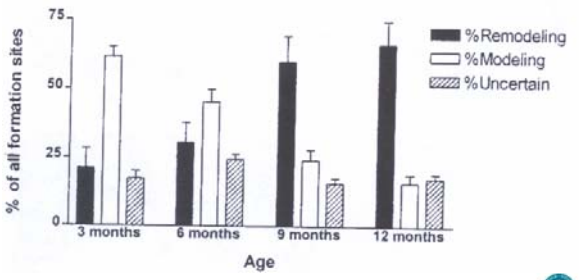
Bone remodeling
- Aged rats or mice or remodeling species

Fractures?
- Bone strength

Bone modeling

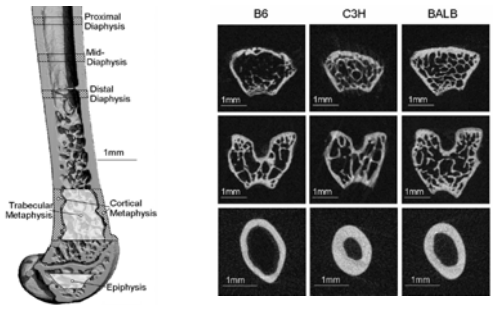


Remodeling sites in rats



Erben The Anatomical Record 1996;246:39-46

Representative cross-sections of C57BL/6, C3H, and BALB mice displaying differential bone quantity and architecture in of the femur



Judex S *et al.* J Bone Miner Res. 2004 Apr;19(4):600-6

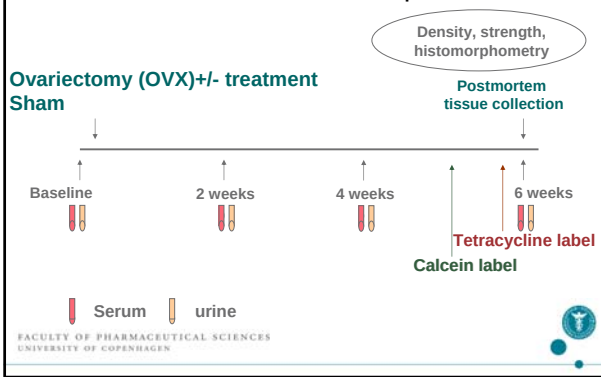
Values for femur mechanical properties determined by bend testing of 29 strains of mice

Strain	Pmax(N)
NOR/Lt	**11.6**
DBA/2J	12.2
C57BL/10J	13.2
NOD/LTJ	13.5
SM/J	13.6
C57BL/6J	**13.7**
RIIIS/J	15.7
A/J	16.1
P/J	16.4
LP/J	17.2
BALB/cByJ	18.0
NZB/B1NJ	18.1
C3H/HeJ	18.3
FVB/NJ	18.7
ST/bJ	19.8
CBA/J	21.8
NON/Lt	22.4
C57L/J	22.5
129/J	**23.1**
C3HSMNSCID	24.2
AKR/J	24.4
KK	28.0
RF/J	**31.3**

C57BL/6J 13.7
129/J 23.1

Wergedal et al. Bone 36 (2005) 111 –122

Study Design
Animal model of osteoporosis



Pre-clinical 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 g/cm3

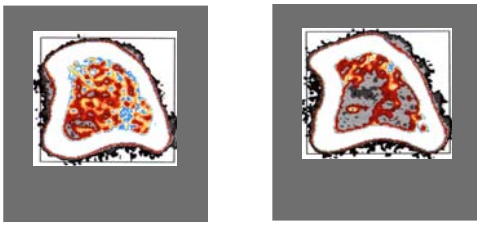
Measurements of Bone Density

Peripheral Quantitative Computed Tomography (pQCT)

BMD (bone mineral density) g/cm3

pQCT

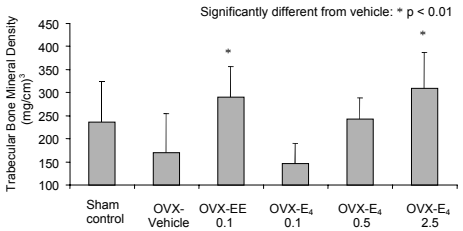
SHAM OVX



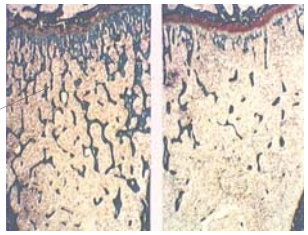
Low High

www.skeletech.com

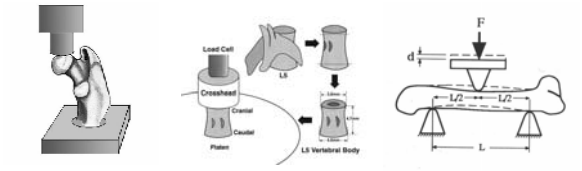
The Ovariectomized Rat Model



Section of rat tibia
bone volume/tissue volume (BV/TV)



Biomechanical Evaluations of bone strength



Compression test of femoral neck

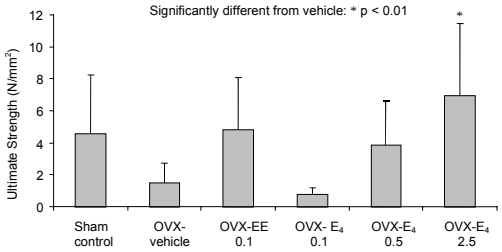
Compression test of lumbar vertebral body

↕ cancellous bone

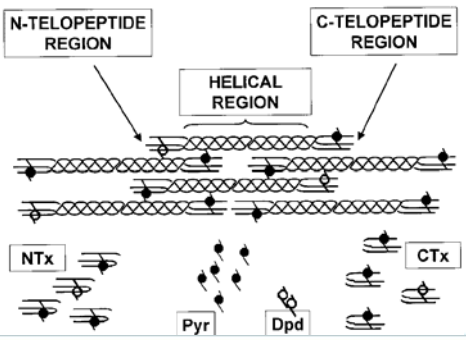
Three-point bending of femoral midshaft

www.skeletech.com

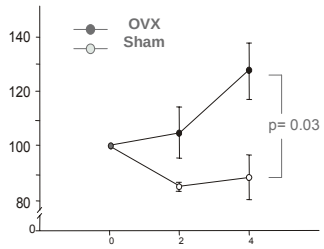
Bone strength



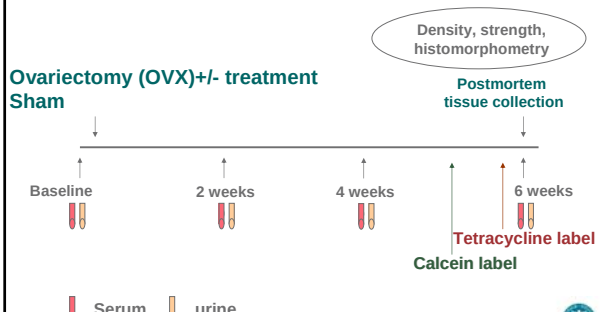
Markers of collagen degradation



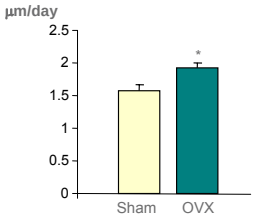
Urinary Deoxypyridinoline/Creatinine
% Change from Baseline

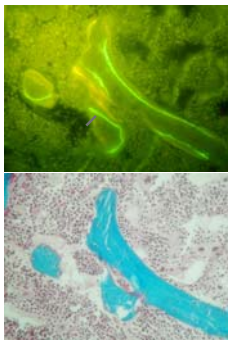


Study Design
Animal model of osteoporosis



Mineral Apposition Rate in the Lumbar Vertebrae





Animal model of bone metastasis

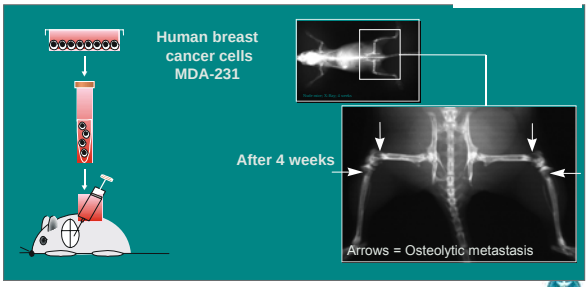
Animal model of bone metastasis

Breast, prostate, lung, multiple myeloma

Syngeneic (mouse to mouse)

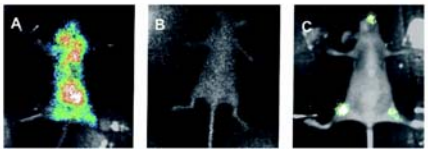
Orthotopic
Induced in breast or prostate tissue
Intracardiac, tail vein
Intrabone injection

Murine model of breast cancer-induced bone metastasis



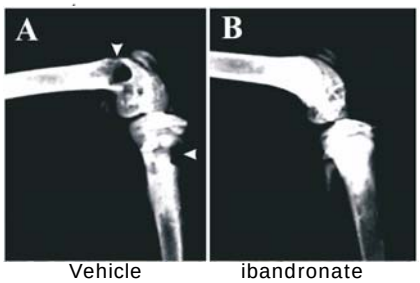
Tracking tumour cells after intracardiac injection in mice

10 min 24 hours 28 days



Wetterwald *et al.* Am J Pathol. 2002; 160:1143

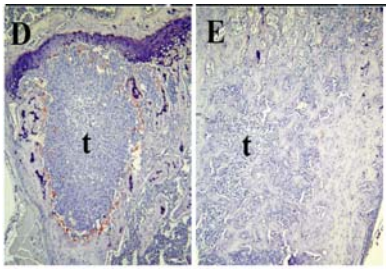
Effect of ibandronate in a mouse model of bone metastasis Osteolysis



Vehicle ibandronate

Amhlaoibh RN *et al.* Clin Exp Metastasis. 2004;21(1):65-74.

Amount of osteolysis does not always correlate with tumor burden



Vehicle Ibandronate

Amhlaoibh RN *et al.* Clin Exp Metastasis. 2004;21(1):65-74.

Arthritis

Rhematoid 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

Kannan *et al*. Pathophysiology 12 (2005) 167–181

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

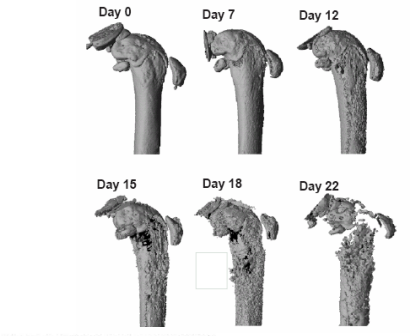
Neuropathic pain

Cancer pain?

The mouse model of bone cancer pain

Bone Destruction

µCT-scanning

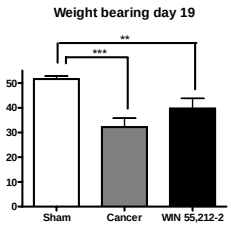


Mouedden & Meert, 2005

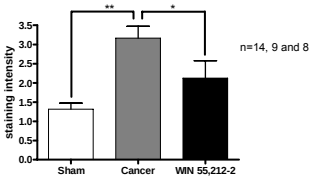
Analysis of Pain-related behaviours

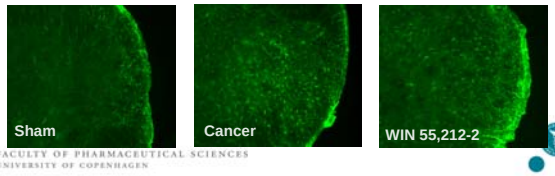
- Threshold for mechanical stimulation (von Frey)
- Limb use (open field and activity induced)
- Number of rearings
- Weight bearing (incapacitance test)
- Number of flinches
- Nocifensive behavior

Weight bearing



GFAP expression in the spinal cord





to consider when doing animal experiments

Species
Strain
Age
Gender
Housing conditions
Chow, water