ANIMAL MODELS OF OSTEOPOROSIS: CRITICAL CONSIDERATIONS

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ANIMAL MODELS OF OSTEOPOROSIS: CRITICAL CONSIDERATIONS (Abstract) Animal models are necessary to study the pathogenic mechanisms of osteoporosis and to test new drugs. The aim of this study was to critically evaluate the experimental models of osteoporosis in rats or mice for an appropriate use in new drug testing. There are five distinct groups of experimental methods for inducing osteoporosis: surgical, immobilization, pharmacological, dietary and transgenic models. Combinations such as ovariectomy plus immobilization or administration of glucocorticoids or diet changes, have the advantage of reducing the time when bone mass loss becomes apparent, especially for cortical bone. No experimental model can reproduce human osteoporosis; therefore, for testing new drugs the selection of a suitable model is essential. Keywords: OSTEOPOROSIS, EXPERIMENTAL MODELS, RAT/MICE, DRUG TESTING.

The multifactorial character of this disease, its incompletely known pathogenesis, the negative impact on the quality of life, with a high incidence of complications and mortality, are arguments for the need of further researches in this field (1, 2). Osteoporosis is characterized by low bone mass and deterioration in bone tissue micro architecture, with a consequent increase in bone fragility and susceptibility to fracture. Affecting both genders, with a higher incidence and prevalence in women, osteoporosis is a progressive systemic skeletal disease with a great social impact, associated with long-term implications, severely decreased quality of life and high mortality. Clinical studies involve high costs and need long periods of surveillance; therefore, experimental animal models play an important role in osteoporosis research, especially for testing new drug treatments (3).

The aim of the study was to critically evaluate the experimental models of osteoporosis in rats or mice for an appropriate use in new drug testing. Data sources were relevant preclinical studies on experimental osteoporosis from PubMed, published in English or French, from 1980 up to March 2015. Searched terms included “experimental osteoporosis”, “rat/mice”, and “biomarker”. From 4,355 papers about experimental osteoporosis published in the interval 1980-2015, there were selected 65% of the original articles and critical
reviews (1,947 papers on rat models and 885 papers on mouse models) describing the methods for inducing osteoporosis and biomarkers. The studies with incomplete data about the criteria for osteoporosis, letters to the editors and commentaries were excluded. The methods used for inducing osteoporosis in rats or mice were: surgical intervention (4-7), immobilization (7-9), drug-induced osteoporosis (10-17), dietary interventions (18-21) and transgenic models (22-24).

**BIOMARKERS IN OSTEOPOROSIS**

Osteodensitometry, a non-invasive method, evaluates the bone loss which occurs immediately after ovariectomy (bone resorption exceeds bone formation); later, bone remodeling reaches a steady state where bone formation and bone resorption are in balance (3). The markers that indicate cancellous bone loss after ovariectomy are: earliest time of bone loss (14 days for the proximal tibial metaphysis, 60 days for lumbar vertebral body, 30 days for femoral neck), time of 50% bone loss (30-60 days for the proximal tibial metaphysis, 180-270 days for lumbar vertebral body and for femoral neck), earliest to achieve steady state (90 days for the proximal tibial metaphysis, 270 days for lumbar vertebral body and for femoral neck), earliest decrease in bone strength (7). The markers that indicate cortical bone loss bone after ovariectomy are the enlargement of the marrow cavity (the site for bone loss analysis is the inner half of the shaft adjacent to the marrow), the earliest changes in the femoral and tibial cortex (90-120 days) and steady state (180 days) (4).

Ovariectomy in mice is not very popular because the markers that point the changes in cortical bone are not clear defined (4). The trabecular and cortical response to ovariectomy are variable among inbred mouse strains, which is in favor of the hypothesis that pre- and postmenopausal bone activity is partly genetically regulated (5).

Comparative with ovariectomy, in the immobilization model the earliest time of cancellous bone loss is 14 days for the proximal tibial metaphysis, 30 days for the distal tibial metaphysis and the time to achieve steady state is 126 days for the proximal tibial metaphysis and 45 days for the distal tibial metaphysis (3). In the immobilization model, the earliest time of bone loss in the cortical bone is 21 days for the femoral cortex and 42 days for the tibial cortex; the time of 10% bone loss is 26 weeks (3).

Other biochemical markers useful in evaluating osteopenia are: blood and urinary levels of phosphorus, calcium, magnesium, markers of bone formation (alkaline phosphatase, osteoclacin) and markers of bone resorption (urinary type I collagen cross-linked N-telopeptides, pyridinoline, tartrate-resistant acid phosphatase).

Histomorphometry, an invasive method, provides information about: number of osteoblasts, osteoclasts, osteocytes, active osteoblasts relative to bone perimeter, trabecular thickness (3).

Mechanical strength testing, an invasive method, assess the mechanical quality of the bone and is applied to diaphyses of long bones (three-point bending, 4-point bending, torsion), vertebrae (compression testing), femoral head (cantilever testing) (3).

**SURGICAL METHODS FOR INDUCING OSTEOPOROSIS**

Bilateral ovariectomy versus orchitectomy is the surgical intervention preferred by most researchers for obtaining osteoporosis (rat, 1691 vs. 99 studies; mice, 491 vs.
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28 studies). Ovariectomy in rats, the most frequently used technique as model for postmenopausal osteoporosis, simulates the clinical criteria of the osteoporotic human skeleton and reproduces the response of therapeutic drugs. Ovariectomy induces androgen deprivation which promotes an osteoporotic model (5).

Alternative interventions in rats for obtaining osteoporosis are hypophysectomy (3 studies), pinealectomy (3 studies) or below-knee amputation (6 studies).

IMMOBILIZATION FOR INDUCING OSTEOPOROSIS

Immobilization for inducing osteoporosis may be realized by non-invasive methods such as: tail suspension (54 studies on rats, 33 studies on mice), confinement (1 study on rats, 1 study on mice) or administration of botulinum toxin (9 studies on rats, 4 studies on mice). Surgical resections of spinal nerve (3 studies on rats), tendon (4 studies on rats) or spinal cord (13 studies on rats, 3 studies on mice) are invasive methods. Immobilization combined with ovariectomy provides a severe bone loss in a shorter period than either method alone (9).

The favorite immobilization model is tail suspension because it causes hypertension and adrenal hypertrophy (6, 7). Botulinum toxin type A-induced immobilization determines similar osteoporotic effects as ovariectomy (8).

DRUG-INDUCED OSTEOPOROSIS

There are several drugs used for inducing osteoporosis (tab. I): corticosteroids (25 studies on rats; 20 studies on mice), gonadotropin-releasing hormone (GnRH) agonists (4 studies on rats) or aromatase inhibitors (1 study on rats). Corticosteroid-induced model of osteoporosis (with Prednisone or Dexamethasone) has a good correlation between drug administration and bone loss (10, 12). The administration of Buserelin, a GnRH agonist, leads to estrogen deprivation, resulting in bone loss similar to bilateral ovariectomy (15). The administration of aromatase inhibitors (Vorozole) in aged male rats induces a net trabecular bone loss in the appendicular and axial skeleton (16).

Association of methods induces osteoporosis more rapidly, using lower doses. One schedule starts 2 weeks after ovariectomy in rats, with dexamethasone 0.3 mg/kg b.w./day once every two weeks (17).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dose</td>
<td>schedule</td>
</tr>
<tr>
<td>Prednisolone (mg/kg)</td>
<td>3.5 mg</td>
<td>s.c., daily, 4 wk</td>
</tr>
<tr>
<td>Dexamethasone (mg/kg)</td>
<td>2.5 mg</td>
<td>i.m., x2/wk, 9 wk</td>
</tr>
<tr>
<td>Buserelin (µg/kg)</td>
<td>25 µg</td>
<td>s.c., daily, 4 wk</td>
</tr>
<tr>
<td>Vorozole (mg/kg)</td>
<td>17 mg</td>
<td>p.o., daily, 15wk</td>
</tr>
</tbody>
</table>

wk: week.

DIETARY CHANGES FOR INDUCING OSTEOPOROSIS

Dietary changes are non-invasive methods less used to obtain osteoporosis in animals: diet deficient in Ca$^{2+}$/vitamin D$_2$/D$_3$ combined with ovariectomy for
achieving osteoporosis more rapidly (18); prolonged Mg\textsuperscript{2+} deficiency (19); high fat diet (20); excessive alcohol (21).

**TRANSGENIC MODELS FOR INDUCING OSTEOPOROSIS**

Transgenic models of osteoporosis (36 studies on rats, 305 studies on mice) are useful for understanding the pathogenesis and for identifying new therapeutic targets for the treatment of osteoporosis. These models use transgenic mice carrying the human genomic region (e.g., TghuRANKL mice carrying receptor activator of NF-kappaB ligand RANKL – a region which plays a key role in osteoclast-induced bone resorption) (22), transgenic mice that inappropriately expresses the cytokines (e.g., IL-4 and IL-13 – involved in the regulation of bone remodeling) (23) or premature accelerated aging in mice (a suitable model for involutional osteoporosis and its treatment) (24).

**CONCLUSIONS**

In our observations we aimed at demonstrating that animal models have a crucial role in osteoporosis research, especially in the development of new therapeutic strategies. Combination of methods (e.g., ovariectomy + immobilization, + glucocorticoid or + diet changes) has the advantage of reducing the time when bone mass loss becomes apparent, especially for cortical bone. There is no ideal animal model for osteoporosis; the selection of an appropriate model is the key to well-done promising researches. Essential in selecting the best model for research and especially for testing new drugs for the treatment of osteoporosis is to know the limits and strengths of various animal models.

**REFERENCES**

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