Experimental Modeling of Osteoporosis in Animals

Abstract. Experimental studies on animals under conditions of osteopenia and osteoporosis modeling significantly expand the view of the mechanisms of primary and secondary osteoporosis development, help determine the effect of various factors affecting the bone tissue, evaluate the effect of medications, new biomaterials, etc. Osteoporosis is a multifactorial disease; its clinical manifestations depend on a complex interplay of environmental, lifestyle and genetic factors. The review of the literature analyzes the data on the use of animals to test the bone strength and quality under various exogenous factors affecting the bone tissue, while the experimental osteoporosis models, one may describe the bone remodeling based on histological, morphometric and biochemical studies, as well as use biomechanical methods to test the bone strength and quality under various triggers of osteoporosis and to determine some pathogenetic pathways of this condition [1, 4-7]. Using experimental models to test the induced osteoporosis one can assess the medication and non-medication efficacy, as their implementation into the clinical practice without using models on animals would be contentious [8, 9].

Furthermore, one may test the bone-implanted biomaterials, various fixative constructions on animals with a modeled osteoporosis in order to study the effect of bone quality on biomaterial restructuring and construction stability. The osteoporosis model replicated on animals under the traumatic bone injury helps evaluating the stage-time parameters of reparative osteogenesis attending this pathology and determining the risk factors resulting in bone nonunion [10].

The above-mentioned factors prove the topicality and importance of replicating such a wide-spread disease as osteoporosis by means of experimental models. Osteoporosis is modeled at various animals: mice, rats, rabbits, dogs, ewes, apes etc. Most experimental protocols, however, involve the laboratory mice and rats as these study
objects are relatively cheap, easily regulated, and capable of replicating the osteoporosis models of various etiology: immobilization, dietary manipulations, hormonal deprivation, harmful substances etc. [1]. In this regard, our review mainly focuses on animal osteoporosis modeling.

The experimental models have a tri-fold value: first and foremost, the experimental replication of this pathology allows detecting a certain pathogenic pathway; secondly, with experimental model, the researcher can use the contemporary methods of studying the molecular, cellular and systemic changes, this fact being impossible with patient’s clinical examination; and, finally, the experimental model of pathological condition is an important object of therapeutic efficacy evaluation for this type of pathology [1].

The animal selection for any study should be based on the following principles: 1) relevance as an analogue; 2) informative value; 3) genetic regularity of the organisms used; 4) background knowledge of biological properties; 5) cost effectiveness and accessibility; 6) summarization of findings; 7) ease and applicability for the experimental manipulations; 8) environmental considerations; 9) ethical and social consequences [11].

One replicates the primary and secondary osteoporosis by means of various methods. By ovariectomy, one induces the Type 1 osteoporosis, i.e. postmenopausal. One can also replicate the secondary osteoporosis, using orchietomy, thyroidectomy, spinal cord or sciatic nerve injury, tenotomy, limb amputation, absence of skeletal loading or zero gravity, chemical substances etc.

**Postmenopausal osteoporosis modeling by ovariectomy**

The most frequently used model of osteoporosis is the postmenopausal one.

The rodent ovariectomy is a procedure of surgical incision of ovaries (Fig. 1). The hormonal changes induced by ovarian extraction result in a reduced estrogen rate, and by consequence an increased negative bone remodeling, provoking bone loss and inducing the bone fragility. Ovariectomy is considered to be the most wide-spread pre-clinical model enabling understanding of menopause-related pathophysiology. This model is also used to develop the new strategies of postmenopausal osteoporosis treatment [12].

Ovariectomy is usually performed at the mature or old rats. The old rats are subject to bone growth termination; usually a few months after ovariectomy one also observes a significant bone loss. However, experiments are most frequently performed at the mature rats, i.e. sexually mature, starting at the age of 3 months old, as they respond to the ovariectomy-induced sexual hormone deficiency in an adequate manner [12].

In order to explore the ovariectomy’s effect on rats, one performs histomorphometric measurements of the frontal sections within a rectangle one millimeter away from the central point of metaphyseal plate in order to avoid the primary osteogenesis site [14]. It was determined that at the trabecular bone the “bone volume/trabecular plus bone marrow (total volume)” (BV/TV, %) ratio diminishes during 30 days after ovariectomy rather quickly, at 50 % rate from the initial value. The comparison of bone loss rate is to be made with control group (mistaken-surgery animals) selected at the beginning of the experiment. With osteoporosis, the bone loss depends on the reduction of number and width of trabeculae. Furthermore, the bone trabeculae separation (Th. Sp) increases due to a growing bone resorption. Next to the reduced bone trabeculae mass, one detects the destructive changes, namely the matrix delamination with collagen fiber imaging, focus of destruction, reduced osteocyte density. Most osteocytes are located in the wide lacunae with irregular edges, signaling the osteocytary osteolysis [15]. The cortical thickness of hip and tibia reduces from periosteum to endostium. Furthermore, the cortex porosity increases due to thickening or fusion of bone channels.

In order to assess the bone remodeling, one selects and calculates the number of osteoblasts as the bone loss occurs under the estrogen deficiency due to an intensified resorption and osteoblast functional disorder [1, 16]. To make precise calculations, it is advisable to measure the response to tartrate resistant acid phosphatase.

However, in order to obtain a full picture of post-ovariectomy osteoporosis from the animal studies, one needs to take into account the specific features of its development, i.e. disproportionate changes of various skeletal sites. One needs to consider this fact while performing other studies, e.g. densitometry [1, 17]. Furthermore, the summarized database of other authors demonstrates that the bone loss due to induced osteoporosis occurs at various stages: in case of proximal metaphysis of tibia it occurs after 14 days, in case of lumbar spine vertebrae it occurs after 60 days, in case of femoral neck it occurs after 30 days. One has obtained data that following the ovariectomy the bone resorption prevails over formation at first, resulting in the overall bone loss. However, very soon the bone remodeling reaches a stationary stage, its resorption and formation matching one another. The bone changes of senile animals after the ovariectomy are similar; they occur during a month or sooner, depending on the skeletal site. By contrast, ovariectomy does not induce bone loss in the long bone epiphyses, distal metaphysis of tibia or tail vertebrae.

Another study also points out the irregularity of bone loss. It determined that ovariectomy results in a reduced bone volume of long bones (their distal metaphysis) but also an increased bone volume of distal metaphysis cortex and medial diaphysis [18]. Along with the cortical porosity, the diaphysary fraction risk grows. The authors drew the conclusion that one factor analysis is not enough to predict the bone fracture risk, as with ovariectomy the bone response changes depending on the bone type and location. The obtained findings expand our knowledge of osteoporosis induced by ovariectomy and its similarity with a postmenopausal one.

Despite the fact that the rat ovariectomy is a “gold standard” of replicated postmenopausal osteoporosis, the use of mice, according to some authors, may be necessary...
for an initial assessment of new osteoporosis medications. The mice require fewer medications; their osteoporosis develops quickly and has some characteristic features [19].

The study of bone markers in the experimental vs. mistakenly-operated animals provides valuable information on bone turnover. Following the ovariectomy, one detects a gradual reduction of alkaline phosphatase (ALP), osteocalcin and PINP rates, reflecting a diminished osteoblast function and bone formation, and a high CTX1 rate, which is a bone resorption marker. According to the obtained findings, PINP and CTX1 rates are recommended benchmarks for bone formation and resorption by the International Osteoporosis Foundation (IOF) and International Federation of Clinical Chemistry (IFCC) [20].

Due to the fact that ovariectomy as osteoporosis model is most thoroughly studied with mice and rats, these animals are most frequently used to assess the medication and non-medication treatment efficacy, bone regeneration disorders as well as bone restructuring with new bio-

Materials used for implants. However, one should note that under the ovariectomy-induced osteoporosis one does not observe any fractures.

Ovariectomy combined with various factors

Ovariectomy combined with glucocorticoids

Having performed the bilateral ovariectomy, the authors recommend an additional Methylprednisolone injection in a daily dose of 1 mg/kg of animal body during 4 weeks. This simple, fast and effective regimen is recommended to induce osteoporosis in rabbits, as it develops faster than with ovariectomy on its own [21].

Another model of ovariectomy combined with glucocorticoid use was replicated in rats. Two weeks after the ovariectomy the rats received a percutaneous Dexamethasone injection in a dose of 0.3 mg/kg once every two weeks [22]. The rats were put out of the experiment after one and three months. The researchers were examining shoulder and hip bone, as well as tibia. They confirmed that combined effect of ovariectomy and Dexamethasone

![Figure 1. Schematic illustration of ovariectomy using a single incision of midline skin (a and b), double dorsolateral skin incision (c) or single ventral transverse incision (d) (adapted from [12])]

Note. X stands for “extraction”. Visual demonstration of ovariectomy is available at PubMed online [13].
studies confirmed replicate this stage adequately. The model of natural age modern perimenopause models involving animals cannot changes. It is the first step to osteoporosis. However, the an’s life when she is subject to a range of physiological to the postmenopausal period Animal models of changes similar as well as ovariectomized animals. The authors concluded as well as Calcium bone rate compared with control rats tivity and Calcium-dependent ATPase (Ca₂⁺-ATPase), hormone blood rates, reduced alkaline phosphatase ac 7 weeks, the rats were swimming in the water cooled down to the postmenopausal period Ovariectomy combined with mechanic unloading of limbs The mature (6-month-old) rats were subject to hind limb unloading following ovariectomy [23]. The mass of hip bone and tibia was measured by dual-energy X-ray absorptiometry (DXA) while the bone strength was measured by means of bending and twisting test. The authors demonstrated that ovariectomy promotes the total BMD reduction of hip bone (-5.5 %) and tibia (-7.3 %) compared to the mistakenly-operated animals. The 4-week use of hind limb unloading on its own did not affect the bone state; however, the combination of two factors — ovariectomy and hind limb unloading — promotes the hip bone BMD reduction (-10.5 %) compared to ovariectomy on its own. The hip bone was more susceptible to this combination of factors than tibia. The BMD of hip bone decreased (-5.3 %) in addition to the total BMD and compared to ovariectomy on its own. The mechanic tests also confirmed the significant reduction of bone strength under those two factors combined. Ovariectomy combined with cold stress Based on the animal model, ovariectomy-induced osteoporosis was tested by cold stress. For 5 minutes during 7 weeks, the rats were swimming in the water cooled down to 8°C [24]. The authors detected endocrine disorders: the blood corticosteroid, thyroxine and thyroid-stimulating hormone blood rates, reduced alkaline phosphatase activity and Calcium-dependent ATPase (Ca²⁺-ATPase), as well as Calcium bone rate compared with control rats as well as ovariectomized animals. The authors concluded that cold stress combined with estrogen deficiency affects the bone tissue negatively. Animal models of changes similar to the postmenopausal period The perimenopause is an important stage in any woman’s life when she is subject to a range of physiological changes. It is the first step to osteoporosis. However, the modern perimenopause models involving animals cannot replicate this stage adequately. The model of natural ageing or the ovariectomy models do not imitate the natural liminal stage, i.e. menopause. Due to this fact, one is continuing to seek models similar to this condition. The estrogen deficiency may be achieved by chemical induction due to ovarian disorders, use of chemical 4-Vinyl-cyclohexene dioxide (VCD) resulting in an early ovarian failure [25, 26]. The in vivo and in vitro studies confirmed that VCD chemical may provoke a selective destruction of rat and mouse primary and secondary follicles due to an accelerated apoptosis imitating perimenopausal stage [27]. The VCD was confirmed to induce otoxicity due to c-kit/kit interference and apoptotic pathways. The VCD effect in rodents promotes a gradual increase of ovarian insufficiency with hormonal and cyclical changes imitating perimenopausal stage. This experimental model may be used to study the early changes of bone tissue under the increasing estrogen deficiency. The VCD-induced animal models provide a certain insight into the female perimenopause, though they are remote from perfect. Osteoporosis modeling by orchiectomy The orchiectomy performed on rodents imitates osteoporosis developing in men. Thus it remains a valuable tool for understanding the androgen deficiency effect on bone [12, 28]. The rodent orchiectomy is presented on Fig. 2. Orchiectomy combined with L-Thyroxine This modeling of secondary osteoporosis in male rats of reproductive age was performed with L-Thyroxine injections in a dose of 25 μg/100 g of body mass during 30 days after orchiectomy [30]. One detected the BMD reduction exceeding the one occurring after orchiectomy on its own. It is possible to obtain the osteoporosis model which is similar to orchiectomy whenever Buserelin, i.e. a synthetic analogue of Gonadotropin-releasing hormone (GnRH) agonist, is used (see below). Modeling senile osteoporosis The identical model of senile (old-age) osteoporosis should involve animals subject to bone loss as a function of their age, resulting in osteopenia and osteoporosis [31]. The animals with a longer life expectancy demonstrate a more pronounced osteoporosis than the animals with a short life expectancy [7]. However, under the laboratory conditions one uses rodents more frequently. First and foremost, these are laboratory mice with a life expectancy of 2–2.5 years which reach their peak bone mass at the age of 4–8 months, and then are subject to a consistent bone loss during ageing, or the laboratory rats with a similar life expectancy. With ageing of these animals, one can detect specific features of bone changes at the tissue, cellular and molecular levels. The senile osteoporotic phenotype is demonstrated by C57BL/6 mice which are subject to the trabecular and cortical bone mass and quality reduction depending on their age [32]. However, there are some distinctions in the compact and trabecular bone loss dynamics of these animals. It was confirmed that the trabecular bone vol-
volume fraction (TBVF) was greatest at the age of 6-8 weeks; however, from week 6 to 24 months it went down. The age-related reduction of trabecular bone volume was more pronounced in female rather than male mice. The cortex thickness increased from 1 to 3 months, and then reduced gradually. The knowledge of specific age-related changes of bone morphology plays an important role in the skeletal response interpretation under various pharmacological and non-pharmacological interventions. Furthermore, one registers a decreased differentiation of mesenchymal stem cells into osteoblasts along with an increased differentiation into adipocytes for C57BL/6 mice, also reflecting on bone quality.

The SAMP6 (Senescence-accelerated mouse-prone 6) line mice made the first model replicated directly to explore the senile osteoporosis [33]. These mice had a specific feature of being susceptible to osteoporosis with spontaneous fractures [34]. Furthermore, the SAMP6 mice have lots of characteristics in common with human senile osteoporosis, namely the vertebral body fractures, while the SAMP8 mice are characterized by sarcopenia and fractures [35]. These data expand the researchers’ scope of tools, and this model becomes important for the study of multifaceted cellular and molecular mechanisms of senile osteoporosis [36].

In order to characterize the mice ageing better, the Jackson Aging Center [37] held a study into the life expectancy of 31 genetically diverse inbred lines. Every 6, 12 and 18 months the researchers measured various body parameters, including bones, enabling calcula-

**Figure 2. Schematic illustration of orchiectomy using a vertical incision of scrotum (a and b) or ventral suprapubic incision (c) (adapted from [12])**

*Note. X stands for “extraction”.*
tions intended for planning and holding research projects.

**Modeling osteoporosis under hypokinesia or microgravity**

One of the methods of osteoporosis inducing in animals is creating hypokinesia conditions. The animal models imitate the situation of zero mechanic stimulation of the body or limbs. For this purpose, one usually uses rodents, i.e. rats or mice.

There are several invasive and non-invasive methods [1]. The non-invasive methods include keeping animals in penal cages, limiting their motions to zero, or bandaging/plastering their hind limbs. Under these conditions, the limb unloading suppresses the bone formation and intensifies the resorption. One models osteoporosis by modeling conditions similar to the ones of space flight, i.e. microgravity.

Immobilization of hind limbs is performed by surgical interventions, i.e. sciatic neurectomy or tenotomy, spinal cord injury. Using surgical methods in order to model osteoporosis under immobilization accelerates the bone loss compared to non-surgical immobilization. The above-mentioned models demonstrate the greatest bone loss in the hind limbs, as the latter are sites of the greatest mechanic unloading. With that, the loss of trabecular bones occurs quicker than that of the compact ones. Immobilization of mice hind limbs during one or two weeks results in a fast decrease of tibia’s trabecular network of up to 50 % compared to the initial level [38]. Unlike ovariection, this model of osteoporosis suppresses the periosteal cortex formation while its endosteal resorption is on the rise.

In order to replicate the immobilization osteoporosis, one suggests Botulinum Toxin Type-A (BTX-A) injections (Botox(r), Allergan, Irvine, California) [39]. The Botulinum Toxin Type-A (BTX-A) is one out of seven neuromuscular blocking agents produced by Clostridium botulinum bacteria. The BTX-A is highly-specific for motor nerve terminals; it has a high capacity for diffusion into muscles after the injection, provoking muscle weakness and paralysis. The BTX-A dose of 8 IU was injected into the right hip bone. The authors compared the left and right hip BMD values at the initial stage and 14 weeks after the BTX-A injection for the control rats and injected rats following ovariection. There were no significant differences between two experimental groups in terms of their BMD values. The authors concluded that this model may be used as an analogue to ovariection for the experimental purposes [39].

**Modeling alimentary osteoporosis**

The model of alimentary calcium deficiency is replicated by keeping animals on a low Calcium grain-legume diet [10]. We have made an experiment involving an experimental sample of rats receiving the following diet from an age of one month: 0.03 % Calcium, 0.029 % Phosphorus, 233 IU Vitamin D, and a control groups receiving the following diet: 1.2 % Calcium (0.8% Phosphorus, 233 IU Vitamin D). According to the histological, ultrasound and morphometric study of trabecular and compact components of hip bone, after the five months of low Calcium diet the examined animals of both sexes demonstrated the rates of bone resorption exceeding the ones of bone formation [40]. However, the morphometric findings demonstrate different rates and mechanisms of bone loss in males and females, and also confirm the estrogen’s dominating role of trabecular bone “protector”, while the androgens are in charge of compact bone protection.

There were other models of alimentary osteoporosis developed. For instance, the experimentally induced Magnesium deficiency results in bone turnover disorders due to an increased expression of anti-inflammatory cytokines. Keeping animals on a high-protein diet disrupts osteogenesis, while the high-lipid diet promotes metabolic syndrome resulting in a secondary osteoporosis.

**Modeling glucocorticoid-induced osteoporosis**

Glucocorticoids induce osteopenia and osteoporosis, which is distinct from postmenopausal or senile one, via bone turnover changes.

The glucocorticoid osteoporosis was induced in 3-month-old male rats by Prednisoloni acetas administration via a peroral tube in a daily dose of 1.5, 3.0 and 6.0 mg/kg [41]. In order to determine the dynamic bone growth parameters, the rats put away from the experiment were injected Tetracycline and Calcein for double-marking of bone restructuring in vivo. Based on a wide range of methods (biochemical, histological and morphometric, mechanical tests, densitometry, micro-CT), the authors detected Prednisolone’s ability to diminish the rat body mass, bone biomarkers in rat’s blood serum and biomechanical parameters to a significant extent. It was found that Prednisolone does not only inhibit the bone formation but also suppresses resorption, resulting in bone strength deterioration. Furthermore, the model proves that glucocorticoids have different effects on cortical and trabecular bones, and on skeletal bones: tibia and hip bone. The young rats induced by glucocorticoids might be used as a model of comparative analysis for the drug’s effect on the bones of minor patients treated by glucocorticoids for a long time.

**Modeling osteoporosis under hypothermia**

Hypothermia is considered one of osteopenia and osteoporosis leading risk factors [42]. In light of this factor, one developed several experimental models exploring the hypothermia effect on bone [43-46].

Slight hypothermia was modeled by keeping rats in a cold chamber (T = -0.2 C) for 5 hours daily during 5 days [44]. The rectal temperature was taken at the experiment’s beginning, after one, three and five hours of cold exposure, as well as during 7 days after the cold exposure was over. Under normal conditions, the rat’s body temperature is 38.5-39.5°C. By the fifth day of the experi-
ment, the young (6-month-old) rat's body temperature decreased by 1.82°C, while the old (24-month-old) rat's body temperature decreased by 4.13°C. Hypothermia caused a negative effect on both trabecular and compact bone remodeling. The surface area of bone trabeculae in the examined 6- and 24-month-old rats was 16.0 to 16.6 % lower than the one of control group animals 28 days after the hypothermia exposure [45]. The osteocyte number of the examined 6-month-old animal trabeculae was also 16.9 % lower than the one of control group. There was a 7.5-fold increase of lacunae devoid of osteocytes. 28 days after the hypothermia exposure, the 24-month-old rats had the number of resorptive cavities full of osteoclasts exceeding the similar number of 6-month-old animals by 33.1 %, while the number of resorptive cavities full of osteoblasts and macrophages was down by 46 %, signaling an imbalance of bone remodeling due to an increased resorption. Hypothermia was negatively affecting the mesenchymal bone marrow stromal cells, suppressing their proliferation and cellular colony formation abilities [44]. After the 7 days of culturing, the number of cellular colonies and their surface area in the bone marrow cultures of the examined 6-month-old animals was lower in comparison to the control group when subjected to the cold exposure (34.5 % and 40.1 %, respectively).

Hypothermia was modeled by a single stint of keeping rats in a cold chamber (-32°C) during 3-4 hours until their rectal temperatures dropped to +12-+13°C, the animals kept alert and active during the entire stint [43]. Having studied the hypothermia’s effect at the first, third and seventh day, one detected diminishing muscle tissue microvessel and hemocapillary lumens, which was a result of edema and pronounced destruction of endothelial cells, dilated venules. The authors claim that these microcirculatory changes may affect the bone tissue due to the developing hypoxia.

Another experimental study replicated hypothermia by forcing rats to swim in cold water for 10 minutes (water’s temperature being 8°C) during 10 days [46]. Upon the experiment’s end, one registered a significant thinning of long bones’ cortical layer.

Ionizing radiation effect on bone

Secondary osteoporosis is induced in animals by means of ionizing radiation, chemotherapeutic agents and chronic hyponatremia.

It was revealed that the young animals subjected to low-dose radiation (0.1, 0.2 or 0.3 Gy) demonstrate an insignificant suppression of growth rate and skeletal bone formation. However, the mature rats tend to develop osteopenia, predominantly of the trabecular bones [47]. Under a dose of 0.1 Gy, the bone trabeculae get shortened by 6.07-7.23 % on average, the primary spongiogenesis density volume decreases by 13.9-14.58 %, the lumbar spine vertebrae demonstrate suppressed osteoplastic processes with a 7-13 % trabecular bone volume reduction. Under a dose of 0.3 Gy, the changes were more pronounced. The low doses of radiation slow down the mineral metabolism, reduce the number of macro- and microelements. The most pronounced signs are observed in a shoulder bone of mature animals where the Calcium and Magnesium rates reach critical values (20-25 %).

The summarized reference data present skeletal changes under the high-dose radiation [48]. For instance, after one dose of 50 Gy, the rats’ tibia demonstrated resorption in the cortex endosteal component (4 week study) along with cortical pore formation between 12 and 15 weeks. After 12 weeks, one reported the decreased bend resistance with an insignificant recovery after 52 weeks.

Other models of osteoporosis

Taking into account the multifactorial nature of osteoporosis, its induction involves chemical substances affecting various metabolic links. However, those substances are barely used.

The prolonged administration of Buserelin, i.e. a synthetic analogue of Gonadotropin-releasing hormone (GnRH) agonist, in a dose of 25 or 75 μg/kg provokes a significant testosteron rate reduction, bone microstructural disorders; however, it does not affect the Calcium bone rate [49]. Similar changes were observed in the group of animals which were subject to orchietomy. There was no difference registered between the biomechanical changes in the examined animals vs. mistakenly-operated animals. However, the administration of Buserelin to female rats resulted in osteopenia, increased bone resorption, reduced Calcium bone rate [50]. The findings testify that sex of the animals is a key factor for the models of osteoporosis induced by various factors.

Transgenic and knockout mice in osteoporosis studies

The transgenic animals obtain new properties due to genetic modification performed by the genetic insertion, deletion or substitution [51]. The obtained genetic changes are transmitted to further generations. The segment inserted into the recombinant double-strand DNA is referred to as a “transgene”.

The osteoporosis model was developed using Klotho mice, a transgenic mouse model, obtained via insertional mutation disrupting the Klotho gene locus. The 8-week-old mice are characterized by the diminished cortex thickness of tibia diaphysis of about 40 %. The histomorphometric studies involving this model showed low bone formation (bone surface covered by osteoblasts up to the bone trabeculae), volume (new-formed bone to a trabecular bone unit per year) and resorption (number of osteoclasts on the bone surface and resorative lacunae’s extension). The histomorphometric findings on bone structural units (BSU) testify to a low bone turnover [52]. A significant decrease of bone formation (80-90 % of control values) is observed, while the bone resorption values are lower (60 %), the fact reminding of human senile osteoporosis. The Klotho mice are considered a valid model to detect the skeletal changes [14].

The transgenic mice were subject to Gamma-Glutamyl Transferase (GGT) test, an enzyme present in the
liver and takes part in the bone turnover. It was revealed that an increased GGT expression functions as a cytokine and stimulates osteoclastogenesis [53].

The macrophage migration inhibitory factor (MIF) is an anti-inflammatory and immunomodulating cytokine which probably participates in the bone turnover; however, the mechanism of its action is unclear. In fact, the transgenic mice with a high MIF expression have an increased bone turnover due to an increased resorption and formation. The MIF resorptive activity is associated with an ability of osteoblasts regulating matrix metalloproteinase secretion, and thus decreasing the bone resorption [54].

The Ki-86 protein plays an important role in a disrupted two-strand DNA reparation. The mutant mice (ki-86-/-) are ageing faster than wild ones; and this ageing reflects as osteopenia on the skeleton.

The knockout mice are the animals whose DNA segment was genetically modified in such a way that certain protein’s expression is absent. 126 knockout mice were created, and they have target gene disorders with an increased rate of anomalous skeletal phenotypes [55]. The use of such animals provides lots of opportunities to detect the disease pathways and to open a new age in osteoporosis studies.

There are numerous endogenous agents involved in the bone turnover, their bone effect being studied. It is well-known that the osteocyte network regulates bone mass acting as mechanosensory cells. Special transgenic BCL2 mice whose osteocyte network is completely disrupted may serve as a model to assess the osteocyte function [16].

One has also created a knockout mice line with a turned-off ageing marker, i.e. Regucalcin (RGN), also known as protein-30, senescence marker protein-30 (SMP30). This protein is predominantly expressed in the liver and kidneys, though it plays an important role in the Calcium homeostasis due to their correlation. SMP30 protects the cells from an excessive Calcium and slows down apoptosis. The knockout mice with a turned-off SMP30 (SMP30 knockout mice) have a low body mass and short life expectancy, their liver accumulating neuro- and phospholipids. The mice developed osteoporosis due to hyperlipidemia and an increased osteoclastic activity paired with osteoblastogenic disorders [56].

The studies of knockout mice also focused on Sox4 gene function. It was revealed that the decreased Sox4 (+/-) protein expression promotes osteoporosis. The study of GPC6 gene function based on the knockout mice enabled analysis into this gene’s changes associated with the BMD changes.

In case of another osteoporosis model being created, the authors relied on the assumption that hypothalamus plays a central role in food consumption and fat mass regulation, as well as in bone mass regulation. The Melanin-concentrating hormone (MCH) expressed by hypothalamus takes part in energy homeostasis regulation [57]. In order to assess the physiological role of this hormone receptor, one has created mice with its absence, MCHRI (-/-). Such animals develop osteoporosis, they have a registered bone mass reduction, predominantly due to the compact bone loss, while the trabecular bone mass did not change. The blood serum C-telopeptide rate, i.e. bone resorption marker in MCHRI (-/-) mice, is significantly elevated signaling a high bone turnover rate. The authors have confirmed that MCHRI signaling promotes an increased bone formation.

At present, there are abnormal skeletal phenotypes of knockout mice associated with 153 priority genes [58]. Among those genes, there are Sox (+/-), whose deficiency provokes senile osteoporosis. One has also confirmed that senile osteoporosis is caused by other gene mutations, i.e. Ab1 (-/-) and SH3BP2(-/-). Osteoporosis, which is similar to the postmenopausal one, provokes Dok1 (-/-), Dok2 (-/-), TRPV5(-/-), BK (-/-), Bcl6 (-/-), MMP14(-/-) mutations etc.

This is why the osteoporosis pathogenesis involves many endogenous and exogenous factors affecting the bone tissue at various directions. The use of animals with an experimentally modelled osteoporosis enables the assessment of specific features concerning various skeletal disorders, duration of pathology formed in the animals, these pathologies being induced by various factors, i.e. ovariectomy, orchietomy, glucocorticoid use etc., allows us to explore the specific remodeling features in the compact and trabecular bones of various skeletal sites. The study of human bone remodeling under various conditions requires bone biopsy, i.e. invasive procedure not always readily accepted by patients. The use of experimental animals to study various factors affecting the bone reflects osteoporotic manifestations occurring in the human bone. There is no perfect osteoporosis model, though the data obtained from animals provide a valuable information on this disease pathogenesis as well as other pathological skeletal changes. The newly developed models of genetically modified animals pave the way to understanding the complex links of osteoporosis pathogenesis and open new vistas in treatment of this grave disease.

Similarities and distinctions of experimental osteoporosis models and pathophysiological changes in subjects affected by osteoporosis

One has performed a comparative analysis of various manifestations of skeletal physiology in human beings and mice [59]. If a human lengthwise bone growth suspends with sexual maturity, the rat slow bone growth occurs even after the sexual maturity (after 3 months), though in combination with a bone loss. The trabecular bone turnover in mice measured at the distal hip bone amounts to about 0.7 %, while the one measured at human iliac crest is about 0.1 % per day. The life cycle of human bone multicellular unit is 6-9 months, while the one of mice is 2 weeks. Another distinction concerns the age-related changes in the trabecular bone architecture of mice and humans. The human subjects experience...
an age-related change of number, thickness and links among the bone trabeculae; with that, there is a greater thinning of trabeculae in men rather than in women [60], while in case of male and female mice (C57BL/6) the number and links of bone trabeculae diminish with age, though their thickness does not change [32]. The rarefaction of trabecular network in mice is much more significant, which may also be attributed to a faster bone turnover, though differentiation disorders and osteoblast life cycle in both humans and mice provoke the resorptive lacunae’s formation, whenever they are not filled by osteoblasts [59]. The cortex changes of mice are similar to the ones of humans, i.e. resorptive cavities are formed, their density increased in the endosteal site. However, the mice ageing (C57BL/6) demonstrates that pores often occupy the greater part of internal cortical part of hip metaphysis in 18-month-old mice, though they go undetected in 6-month-old mice. Despite the increased body mass and skeletal BMD, C57BL/6J mice demonstrate the age-related decrease of vertebral and distal hip volume (trabecular substance) which occurred early and last for their entire life. These changes are more pronounced in female rather than male rats. Understanding the age-related changes of bone morphology is also required to interpret bone tissue response to the pharmacological intervention or genetic manipulations in mice [32].

Overall likes were detected in the vertebral trabecular microstructures of both humans and mice. The vertebral changes are extremely disparate in the entire skeleton, while the trabecular network density reduction in SAMP6 is fast to develop in the caudally located vertebral bodies than in the cranial ones. Similar phenomena are also observed in humans [34]. These conclusions underscore the relevance of SAMP6 line use in order to explore the vertebral fragility associated with a senile osteoporosis.

There are other common links in the age-related bone loss mechanisms which concern both humans and mice. For instance, it is the oxidative stress and a related activation of FOXO transcriptional factors, collateral hyperglycocorticisim and an increased lipid oxidation [41, 61].

One should note that there are also distinctions in rat skeletal area changes and in women’s bones. The latter are subject to the first and gravest changes predominantly in the trabecular substance of distal hip bone and proximal tibia. In this regard, in order to confirm the development of animal osteoporosis one examines the above-mentioned sites. It was revealed that the distal tibia metaphysis of rats is similar in terms of its architecture to the one of adult people. There is another distinction: ovariectomized rats do not suffer from fractures which are similar for the ones of postmenopausal women.

However, considering the positive aspects of experimental models replicated on the rats and mice, they have found a wide-range application in fundamental osteoporosis studies.

Methods of bone assessment under osteoporosis modeling

The methods used to assess the bone mass, architecture and bone turnover in animals with a modelled osteopenia and osteoporosis are similar to the ones used for similar purposes in humans, though in animals this range is much wider [1, 19]. Both in animals and humans, one measures the biochemical marker rates in blood and urine, i.e. Calcium, Phosphorus, Magnesium, as well as specific proteins secreted by osteoblasts and osteoclasts while remodeling, i.e. bone formation markers, such as alkaline phosphatase and osteocalcin, and bone resorption markers, such as Tartrate-resistant acid phosphatase (TRAP or TRAPase), N- or C-telopeptide of Type 1 collagen.

The modern bone densitometers help us measure BMD in animals with a modelled osteoporosis [1]. The dual-energy X-ray absorptiometry (DXA) is frequently performed to assess BMD and bone mineral content (BMC) of animal’s total skeleton as well as certain sites. Its use is possible in small animals: mice and rats. Peripheral quantitative computer tomography (pQCT) has certain advantages over DXA as this method allows analyzing trabecular and cortical bones on their own. Although DXA and pQCT both provide important information on BMD and fracture risk, histology and morphometry are of an equal value. They are assessing compact and trabecular bone architecture, trabecular volume, osteoblast, osteoclast and osteocyte numbers, as well as determining dynamic parameters of bone formation, i.e. expanding our scope of knowledge of bone remodeling under osteoporosis induced by other factors.

The experimental animal models are used to perform the biomechanical studies. In order to assess the mechanical properties, they examine long bones by bending them in 3-4 places and performing the twisting tests. The compression test or a combined bending-and-compression test may be applied to assess the vertebrae and femoral neck.

The experimental osteoporosis models replicated on animals and involving multiple study methods expand our knowledge of pathology development mechanisms, bone turnover changes and specific modifications affecting the bone and human body.

Bioethical regulations of experimental animal use

A key component of animal-involving experimental projects is a concerted plan of experiments with the Bioethics Committee of the establishment holding the studies. The medicobiological assays on animals are performed under the aseptic conditions and in compliance with the European Convention, the Law of Ukraine on the Humane Treatment of Experimental Animals, and also in compliance with the universally accepted international standards based on the “three Rs” [62-64]: refinement, i.e. humanization of experiments (alleviation of pain, use of anesthetics during surgery and euthanasia etc.); reduction, i.e. choosing the optimum number, type
and age of experimental animals; choosing an adequate and correct model; substantiating the objective study methods; replacement of experimental animals with alternative models, wide use of in vitro studies etc.

The animals are put out of the experiment only under anesthesia.

Conclusions

The experimental animal studies under the modeled osteopenia and osteoporosis conditions expands our understanding of primary and secondary osteoporosis, allows us to study various factors negatively affecting the bone tissue, to assess the drug and new biomaterial effect. Osteoporosis is a multifaceted disease, its clinical manifestations reflecting a complex interaction of environmental factors, lifestyle and genetic factors. The newest achievements in the field of osteoporosis modeling are the transgenic and knockout mice, producing models which help us study the components of genetic disorders, potentiating the new methods of treatment and prevention of this grave pathology.

References


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Резюме. Експериментальне дослідження на тваринах в умовах моделювання остеопенії й остеопорозу значно розширює погляд на механізми розвитку першого і вторинного остеопорозу, дає змогу визначити вплив різних факторів, які по- рушують стан кісткової тканини, оцінити дію медикаментозних засобів, нових біоматеріалів тощо. Остеопороз є багатофакторним захворюванням, його клінічні прояви залежать від складного взаємозв’язку факторів довкілля, способу життя та генетичних факторів. В огляді літератури проаналізовані дані щодо використання тварин для вивчення особливостей перебігу остеопорозу за моделювання хірургічними та нехірургічними методами цієї патології. Основними досягненнями у моделюванні остеопорозу є створення трансгенних і нокаутних мишей, на моделях яких можливо виявити складові генетичних уражень, що, безумовно, сприятиме розробленню нових методів профілактики та терапії цієї тяжкої патології. Відзначено схожість і відмінності експериментальних моделей остеопорозу до патофізіологічних змін у людини внаслідок остеопорозу. Зроблено акцент на біоетичних нормах роботи з експериментальними тваринами.

Ключові слова: остеопороз; остеопенія; моделювання на тваринах; щури; миші; патоморфологія кістки