EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 4
81-85
Year: 2022

EXPERIMENTAL OSTEOPOROSIS: DIFFERENT METHODS AND SPECIAL EMPHASIS ON GLUCOCORTICOID-INDUCED OSTEOPOROSIS

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Article Received on 13/01/2022 **Article Revised on 03/03/2022** **Article Accepted on 24/03/2022**

ABSTRACT
Osteoporosis (OP) is the most common systemic skeletal disease characterized by progressive bone loss and increase fragility fractures, it affects commonly the old population and females more than males. It's a worldwide challenge as OP affects more than 200 million people, in addition, it impacts a great economic burden in the aging population due to increased dependence and mortality. Glucocorticoid-induced osteoporosis (GIOP) is the most common secondary OP, that was observed with their prolonged use in the treatment of autoimmune disease, neoplasms, and organ transplantation. Designing a proper experimental model of OP is necessary to understand the pathological mechanisms and to evaluate the efficacy of new drugs. There are different methods for induction of experimental OP including: pharmacological, surgical, immobilization, diet, and transgenic. Also, combinations of these methods have the advantage of reducing the time needed for induction of OP and ensuring an apparent OP model.

INTRODUCTION
Osteoporosis (OP) is a systemic skeletal disease evidenced by decreased bone density and microarchitectural degradation of bone tissue, resulting in increased bone fragility.[1] It is also described as a bone mineral density (BMD) that is more than 2.5 standard deviations (SD) units below the mean BMD value for a young adult, according to the World Health Organization (WHO).[2] O.P. is a major health issue that has an impact on people's quality of life because it is linked to morbidity and death, as well as a financial burden.[3]

Primary OP includes postmenopausal OP, which is caused by an estrogen deficit, and senile OP, which is caused by bone mass loss owing to the aging of cortical and trabecular bones.[4] Secondary OP occurs as a result of the existence of particular diseases, lifestyle factors, and medication use that cause bone loss.[5]

An OP model is necessary to analyze new medicine efficacy, duration of the pathogenic process, and pathways.[6],[7] Because there are various limitations to creating an OP model in humans, a reliable OP induction approach in animals is required. Tobacco use and dietary habits make it difficult for scientists to research in a homogeneous community. A better understanding of OP and expressing the unknown elements of the disease can be achieved by producing osteoporotic animal models.[8]

However, OP may be easily based on a variety of species, but choosing the right model is crucial.[9] The following criteria should be used to pick animals for every study: they must meet national and local ethical standards, be conveniently accessible to experimental centers, be safe to handle, cost-effective, and reproduce reliably. The majority of these criteria are met by laboratory mice and rats.[10] Furthermore, rodents develop rapidly, have a short lifespan, and have good skeletal qualities. Furthermore, rodents share many characteristics with humans, including their genomes.[11] However, the majority of experimental techniques use laboratory mice and rats, which are capable of mimicking OP models of diverse etiologies such as immobilization, nutritional manipulations, hormone deprivation, and drug-induced OP.[7]

Method of induction of osteoporosis
1. Drug-induced osteoporosis
The drugs used to induce OP include corticosteroids, gonadotropin-releasing hormone agonists, aromatase inhibitors, and retinoic acid. Drug administration and bone loss are closely linked with prednisone or dexamethasone-induced osteoporosis (GIOP).[12] A gonadotropin-releasing hormone (GnRH) agonist, buserelin, promotes estrogen insufficiency, which causes bone loss similar to bilateral ovariectomy.[13] Aromatase inhibitors (Vorozole) cause net trabecular bone loss in the appendicular and axial skeletons of aged male rats.[14]

Because of its short modeling time, retinoic acid, a vitamin A derivative, is a regularly utilized modeling approach for acute OP in rats. Oral treatment of 70–105 mg/kg retinoic acid for two weeks can successfully produce an OP model.[15] Retinoic acid can activate osteoclasts and increase bone resorption, but it does not affect bone production or the mineralization process of the bone matrix, and it does not impede the activity of osteoblasts. As a result, bone remodeling is out of equilibrium, with bone resorption exceeding bone production, eventually leading to OP in animals.[16] This model has the benefits of being simple to use, having a high success rate, and having typical symptoms. This model is extensively utilized in the research and development of new medications because it has the advantages of ease of use, a high success rate, and typical symptoms.[15]

Glucocorticoid induced osteoporosis (GIOP)

GIOP is the most prevalent iatrogenic cause of secondary OP and the most common cause of the secondary OP. The most prevalent significant GIOP-related adverse event is fragility fractures. When bone mineral density (BMD) is quickly decreasing following exposure to glucocorticoids (GCs), asymptomatic fractures might occur.[17] Vertebral fractures are more common in those with GIOP, but hip fractures are also more common. Trabecular bone is more affected by bone loss than cortical bone.[18]

Pathophysiology of GCs induced osteoporosis

GIOP's pathophysiology is characterized by poor or decreased bone production over time, as well as an increase in bone resorption in the early stages (the first year after starting treatment). Osteoblastogenesis is inhibited by GCs, which cause death in osteoblasts and osteocytes while extending the lifespan of osteoclasts.[19] The role of autophagy and its control by GCs in bone cells, particularly osteoblasts, has gotten a lot of attention in recent years.[20]

Osteoblasts

Peroxisome proliferator-activated receptor 2 (PPAR2), Kruppel-like factor 15 (KLF15), CCAAT/enhancer-binding protein- (C/EBP), adipocyte protein 2 (aP2), and canonical WNT signaling are all affected directly by GCs.[21] [22] GCs upregulate PPAR2, KLF15, C/EBP, and aP2, causing pluripotent precursor cells to preferentially differentiate into adipocytes rather than osteoblasts, reducing the number of osteoblasts. In the WNT–-catenin signaling pathway, GCs repress WNT16 expression while increasing sclerostin and other pathway inhibitors in a time- and dose-dependent manner, resulting in impaired osteoblastogenesis and bone loss,[23] [24]

Interstitial collagenase (the enzyme that destroys type I collagen) synthesis in osteoblasts is also increased by GCs via post-transcriptional mechanisms.[25] Furthermore, GCs impact osteoblast autophagy[20] in a dose-dependent manner; hence, they appear to promote autophagy and sustain osteoblast viability and function at low or physiological concentrations. High doses, on the other hand, inhibit the process, resulting in a decrease of osteoblast-associated gene expression and an increase in apoptosis.[26]

Complex effects on signaling pathways mediated by the Notch receptor have also been found in osteoblasts and osteocytes. Notch expression is increased by GCs, which leads to increased expression of Notch target genes that encode repressive transcription factors such HES and HEY, which may contribute to altered osteoblast function and decreased bone formation.[27] GCs, on the other hand, diminish HEY expression by inhibiting transcription, a mechanism that may preserve or increase bone mass at normal glucocorticoid concentrations.[28]

Osteoclasts

The effects of GCs on osteoblasts cause a rise in the RANKL to osteoprotegerin ratio, which leads to increased bone resorption by boosting osteoclast development and maturation. This mechanism, as well as additional impacts on IL-6[29] and interferon expression, could explain the transitory increase in bone resorption observed in individuals starting glucocorticoid medication. The long-term effects of GCs on osteoclast function, on the other hand, are less obvious; some studies suggest that GCs can alter the osteoclast cytoskeleton, resulting in decreased osteoclast activity despite increasing their lifespan.[30]

Osteocytes

The role of osteocytes in regulating bone remodeling and integrating mechanical and hormonal cues to modulate the activity of osteoblasts and osteoclasts is now well established.[31] Aside from inducing osteocyte death, GCs have been shown to have several additional effects. GCs, for example, appear to alter the elastic modulus of bone tissue near osteocyte lacunae.[32] Fluid flow disruption in the osteocyte–canalicular network may also contribute to changes in the surrounding bone's material properties. Furthermore, GCs cause osteocytes to produce sclerostin and Dickkopf-related protein 1 (DKK1)[33], which impair osteoblast function by inhibiting WNT signaling. Furthermore, osteocyte apoptosis is linked to skeletal vascularity, angiogenesis, hydration, and strength[34], all of which could be contributory factors to fracture risk.

These glucocorticoid actions on bone cells are suggested to explain the GIOP's early and long-term stages. Increased bone resorption and fast bone loss resulting from the first stimulation of RANKL production by existing osteoblasts and osteocytes. Meanwhile, lower osteoblastogenesis and increased death of osteoblasts and osteocytes diminish bone production, which leads to a reduction in osteoclastogenesis signals in the long run.

Furthermore, through decreasing transmembrane transport and intestinal absorption of calcium, GCs

reduced blood calcium levels and increased calcium excretion. Negative calcium balance can increase blood phosphorus levels by increasing phosphorus release from the bone matrix into the blood, as well as calcium release from the bone matrix into the blood.[35] These glucocorticoid-induced alterations can accelerate bone resorption, causing alkaline phosphatase activity to rise in response, promoting bone growth.[36]

Several GCs have been tested in animal models of GIOP, according to a study done by Xavier et al.2022. Although dexamethasone is the most commonly used corticosteroid in rats and prednisolone is the most commonly used corticosteroid in mice, it is difficult to accept them as reference GCs in OP animal models.[37] Dexamethasone was given to rats at the lowest dose of 0.1 mg/kg daily for 60 days and the highest dose of 25 mg/kg twice per week for six weeks.[38].[39] The lowest dose of prednisone was 1.5 mg/kg per day for 90 days, and the highest dose was 6 mg/kg per day for 90 weeks.[40]

Ren and his colleagues (2015) reported that dexamethasone is the most potent inductor of OP, among other corticosteroids, in animal models; moreover, it is characterized by its long-term action thus avoiding the need for daily injections.[41] This was agreed by Wood and his coauthors (2018) who stated that this dosage and interval of dexamethasone administration was documented to induce generalized OP.[42]

2. Surgical induced osteoporosis

The surgical intervention favoured by most studies for obtaining OP in female and male rats is bilateral ovariectomy versus orchiectomy. The most commonly used approach as a model for postmenopausal osteoporosis is ovariectomy in rats, which imitate the clinical criteria of an osteoporotic human skeleton and the response to therapeutic drugs. Orchiectomy promotes androgen deficiency, resulting in an osteoporotic model.[43]

Hypophysectomy, pinealectomy, or below-knee amputation are one of surgical options for obtaining OP in rats.[44]

3. Immobilization-induced osteoporosis

Immobilization for inducing OP is considered as non-invasive methods such as tail suspension, confinement, elastic bandaging[45], or botulinum toxin administration.. When immobilisation is combined with ovariectomy, extensive bone loss is achieved in a smaller duration of time than when either procedure is used alone.[46]

Tail suspension is the most popular immobilisation type because it induces hypertension and adrenal hypertrophy.[8] The osteoporotic consequences of botulinum toxin type A-induced immobility are similar to those of ovariectomy.[47] The increase in osteoclast activity and osteoclastogenesis that occurs after botulinum toxin-induced immobility has been documented, and these two factors are thought to play critical role in the fast bone loss seen in this form of immobilization.[48]

Immobilization-induced bone loss is characterised by decreased bone mineral density, impaired trabecular microarchitecture, and a higher risk of complicated fractures as a result.[49]

3. Dietary changes for inducing osteoporosis

Dietary changes are non-invasive methods less commonly used to induce OP in experimental animals: diet deficient in Ca2+/vitamin D2/D3 combined with ovariectomy for achieving OP more rapidly.[50] Also, prolonged Mg2+ deficiency; a high-fat diet; excessive alcohol may induce OP.[44]

4. Transgenic models for inducing osteoporosis

Identifying the pathophysiology of OP and exploring new therapeutic targets for OP therapy are both aided by transgenic models of the disease. TghuRANKL mice expressing receptor activator of NFkappaB ligand RANKL, a gene that plays a major role in osteoclast-induced bone resorption, are used in these experiments.[51] Transgenic mice that generate premature accelerated ageing in mice (a suitable model for involutional OP and its therapy) or abnormally express cytokines (IL-4 and IL-13, which are involved in the regulation of bone remodelling).[52]

CONCLUSION

GCs are widely and successfully used as an immunosuppressive medication in a variety of inflammatory illnesses. Bone loss has been associated to long-term or high-dose glucocorticoid use, leading in GIOP and increased fracture risk. While the processes are complicated, they include an increase in osteoclast function early in glucocorticoid usage, followed by a decrease in bone formation due to osteoblast and osteocyte apoptosis. In addition to other techniques, glucocorticoids are regarded a very essential method of inducing OP in experimental animals. Furthermore, GIOP in rats is similar to human bone remodelling, which contributes to the high repeatability of rat models employed in the development of new medications for the treatment of OP.

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