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Review Article

The molecular etiology and treatment of glucocorticoid-induced osteoporosis

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Abstract

Glucocorticoid-induced osteoporosis (GIOP) is the most common form of secondary osteoporosis, accounting for 20% of osteoporosis diagnoses. Using glucocorticoids for >6 months leads to osteoporosis in 50% of patients, resulting in an increased risk of fracture and death. Osteoblasts, osteocytes, and osteoclasts work together to maintain bone homeostasis. When bone formation and resorption are out of balance, abnormalities in bone structure or function may occur. Excess glucocorticoids disrupt the bone homeostasis by promoting osteoclast formation and prolonging osteoclasts' lifespan, leading to an increase in bone resorption. On the other hand, glucocorticoids inhibit osteoblasts' formation and facilitate apoptosis of osteoblasts and osteocytes, resulting in a reduction of bone formation. Several signaling pathways, signaling modulators, endocrines, and cytokines are involved in the molecular etiology of GIOP. Clinically, adults ≥40 years of age using glucocorticoids chronically with a high fracture risk are considered to have medical intervention. In addition to vitamin D and calcium tablet supplementations, the major therapeutic options approved for GIOP treatment include antiresorption drug bisphosphonates, parathyroid hormone N-terminal fragment teriparatide, and the monoclonal antibody denosumab. The selective estrogen receptor modulator can only be used under specific condition for postmenopausal women who have GIOP but fail to the regular GIOP treatment or have specific therapeutic contraindications. In this review, we focus on the molecular etiology of GIOP and the molecular pharmacology of the therapeutic drugs used for GIOP treatment.

Keywords: *Bone remodeling, Glucocorticoid, Osteoblast, Osteoclast, Secondary osteoporosis*

Introduction

There are >49 million patients with osteoporosis in developed countries, such as the United States, European Union, Australia, and Japan [1]. Patients with osteoporosis tend to develop vertebrae and hip fractures. Vertebrae fractures and fragility fractures at other sites of the body have increased by millions with the population of osteoporosis [2-5], which causes a heavy financial burden on the country [2,6]. Moreover, complications may arise in addition to pain and limited mobility, which increases the risk of death in fracture patients and imposes financial burdens on the family and society [7,8]. Therefore, several countries recognize osteoporosis as a major public health issue, and the World Health Organization has ranked osteoporosis as the second most crucial health care issue worldwide. Osteoporosis can be divided into (1) primary osteoporosis (including postmenopausal osteoporosis and senile osteoporosis) and (2) secondary osteoporosis. Primary osteoporosis is most common in postmenopausal women [9-11] and elderly persons [12]. Secondary osteoporosis has been associated with various congenital diseases and endocrine disharmony, as well as nutritional status and some medications [13]. The most common form of secondary osteoporosis is glucocorticoid-induced osteoporosis (GIOP) [14], accounting for 20% of all forms of osteoporosis [15]. The majority of these patients have autoimmune diseases (e.g., rheumatoid arthritis and lupus erythematosus), allergic diseases (e.g., asthma and atopic dermatitis), or have undergone organ transplantation. GIOP occurs in two phases: an early phase in which bone mineral density (BMD) declines due to rapid bone resorption and a slow and progressive phase in which BMD declines due to the impaired bone formation [16]. The underlying mechanism of GIOP could be complicated and multifactorial. In this review, we provide an overview of the molecular etiology, assessment,

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and treatment options in the aspect of molecular pharmacology for GIOP.

Endogenous glucocorticoid is required for bone homeostasis

Bone remodeling is a normal physiological process that involves bone resorption and bone synthesis. Under normal physiological conditions, bone resorption and bone formation are in balance, and many cytokines, hormones, and signaling pathways are involved [17] [Figure 1]. The bone remodeling process undergoes continuously during which osteoclasts absorb aged or damaged bones, whereas osteoblasts and osteocytes are responsible for new bone formation. However, if an imbalance arises, abnormalities in the bone structure or function may occur, resulting in osteometabolic disorders, such as osteopetrosis or osteoporosis [18]. Osteoblasts, osteocytes, and osteoclasts interplay with each other to maintain bone microstructure and homeostasis. Osteoblasts and osteocytes secrete receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) to regulate osteoclasts proliferation and differentiation [16]. On the other way, the activated transforming growth factor-beta (TGF-β) and bone morphogenetic protein (BMP) released from the bone matrix after bone resorption also regulate osteoblasts formation [19,20]. Moreover, osteoblasts and osteocytes negatively feedback the differentiation of osteoblasts by inhibiting Wingless-related integration site (WNT) signaling through the secretion of WNT antagonists, Sclerostin (SOST), and Dickkopf 1 (DKK1) [21].

Endogenous glucocorticoid at physiologic concentrations is necessary for osteoblasts to maintain bone homeostasis [22,23]. The physiological activity of glucocorticoids is regulated by two enzymes, namely 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and type 2 (11β-HSD2), among which 11β-HSD1 activates glucocorticoid, whereas 11β-HSD2 inactivates glucocorticoid [24]. Studies using mouse models elucidate the significance of endogenous glucocorticoids in bone homeostasis. The decrease of glucocorticoid sensitivity in osteoblasts by transgenic expressing of glucocorticoid inactivating enzyme 11β-HSD2 causes a reduction of the bone mass [25,26]. Mice with conditional knockout of the glucocorticoid receptor in osteoblast lineage also reveal a significant reduction of vertebral bone density and osteoblast activity [27]. These results suggest that endogenous glucocorticoid is necessary for osteoblast activity and bone mineralization. In another way, human diseases causing an imbalance of endogenous glucocorticoid secretion also impair bone metabolism. Cushing's disease, causing an elevation of serum level of endogenous glucocorticoids, is correlated with osteoporosis [28-30]. Patients with Addison's disease who have a reduced serum level of endogenous glucocorticoids are also associated with a higher risk of hip fracture [31]. In conclusion, evidence from animal models and clinical observations suggests an essential role of endogenous glucocorticoid in maintaining bone remodeling. While the proper regulation of glucocorticoids' physiological concentration is essential for bone homeostasis, excessive

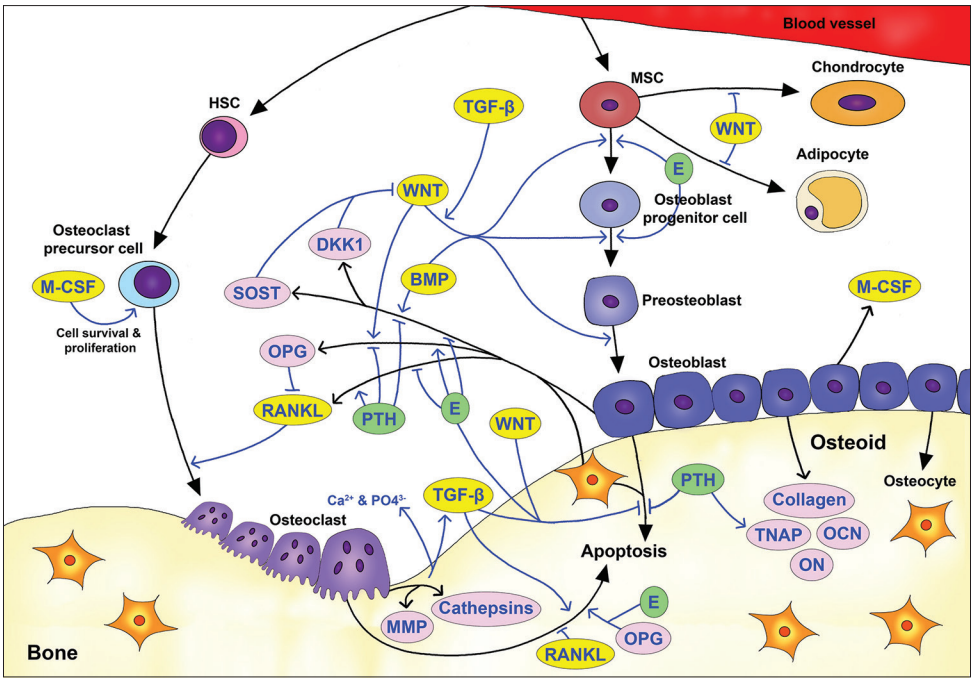

Figure 1: Schematic representation of signaling pathways involved in bone remodeling and the formation of osteoblast and osteoclast. WNT, transforming growth factor-beta, bone morphogenetic protein, parathyroid hormone, and estrogen (e) are essential modulators of osteoblast and osteoclast formation. WNT and bone morphogenetic protein enhance the differentiation of osteoblasts. Bone morphogenetic protein, estrogen, and parathyroid hormone could indirectly regulate WNT activity by controlling the expression of *Sost*, and *Dkk1* from osteoblasts and osteocytes. Transforming growth factor-beta enhances bone formation by suppressing the apoptosis of osteoblasts and osteocytes and enhancing the apoptosis of osteoclasts. Moreover, estrogen and WNT also suppress the apoptosis of osteoblasts and osteocytes. Blue lines indicate the effects of signaling molecules or the secreted proteins on the regulation of bone remodeling. Ligands are marked as yellow ovals. Signal modulators or the extracellular matrix proteins are marked as pink ovals. Endocrines are marked as green ovals

glucocorticoids cause bone loss through the dysregulation of osteoblastogenesis and osteoclastogenesis [Figure 2].

The negative impact of excessive glucocorticoids on osteoblast and osteocyte

The therapeutic concentration of glucocorticoids reduces the formation and survival of osteoblast and osteocyte. Osteoblasts are differentiated from mesenchymal stem cells (MSCs) which travel through the blood vessel to reach the bone surface [32]. At the bone surface, the WNT signaling promotes the differentiation of MSC into osteoblast progenitor cell [33] and inhibits the differentiation of MSC into chondrocyte or adipocyte [34,35]. In the modulation of osteogenesis, glucocorticoids facilitate the differentiation of MSCs into adipocytes instead of osteoblast progenitor cells [36-38].

The differentiation of osteoblast progenitor cells into preosteoblasts and then osteoblasts requires the action of *WNT* and BMP signaling [39-41] by which activate the expression of *Runt-related transcription factor 2* (*Runx2*) *and Osterix* (*SP7*) transcription factors [42,43]. Accordingly, excess glucocorticoids exposure suppresses WNT signaling by decreasing Wnt expression [44], bolstering the expression of WNT antagonists, such as *Dkk1* [22,45-47], *Sost* [46,48], and *Secreted frizzled-related protein-1* (*sFRP-1*) [22,49], and increasing the expression of negative WNT signaling regulator *Axin-2* [49]. It is to be noted that the serum concentration of SOST is reduced in humans, which might reflect a compensatory mechanism that remains elucidated [50,51]. Glucocorticoids also suppress the BMP signaling by inhibiting BMP-2 expression [46,52] and enhancing the expression of BMP antagonists – *Follistatin* and *Dan* [49]. Besides, glucocorticoids suppress both the expression of *Runx2* and RUNX2 activity and thus inhibit osteoblast maturation [53,54].

In addition to WNT and BMP, TGF-β is also involved in regulating osteoblast formation. TGF-β could promote the differentiation of osteoblast progenitor cells from MSCs [55] by enhancing the WNT signaling [56]. On the other hand, TGF-β inhibits osteoblasts and osteocytes' differentiation by decreasing the expression of *Runx2* [57-62]. However, the essentiality of TGF-β in the regulation of osteoblastogenesis can be evident by the study showing that *Tgfb1*-null mice exhibit a significant loss of trabecular bone density and the reduction of osteoblasts [63]. Even limited literature addresses glucocorticoids' effect on TGF-β signaling; it has been reported that glucocorticoid treatment decreases the mRNA level of *TGF-β* [64].

Excess glucocorticoids also lead to apoptosis of osteoblasts and osteocytes. The undifferentiated osteoblast usually goes through apoptosis a few months after its formation. WNT [65], TGF-β [66,67], interleukin-6 (IL-6) [67], and estrogen [68-70] are reported to suppress the apoptosis of osteoblast. By contrast to osteoblasts' 3-month lifespan, osteocytes are long-lived bone cells that can survive for more than

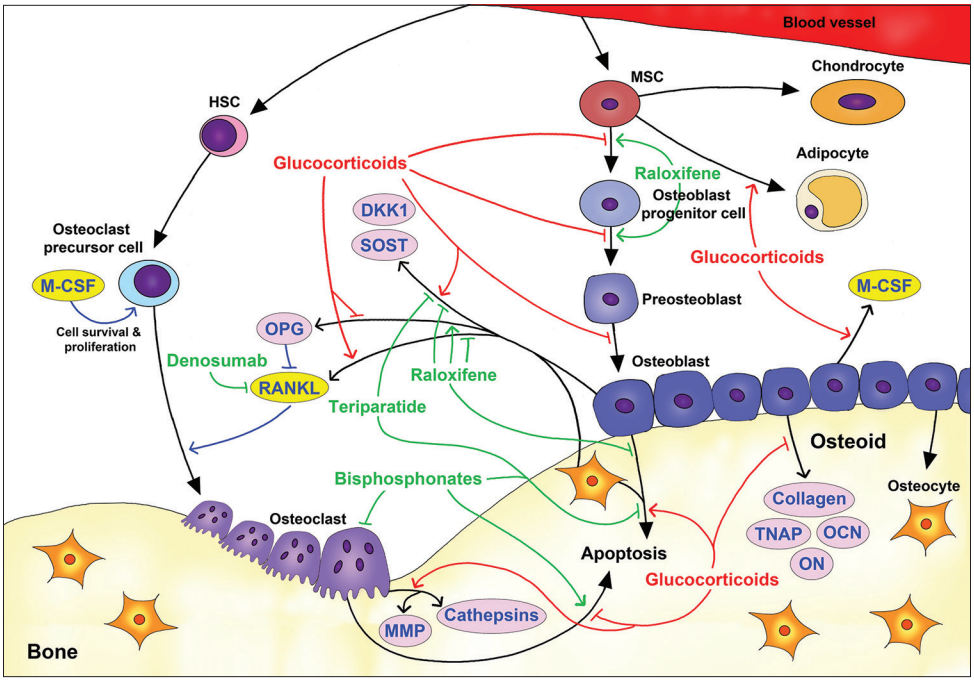

Figure 2: Schematic representation of the molecular etiology of glucocorticoid-induced osteoporosis and the effect of anti-osteoporotic drugs. Glucocorticoids (red) induce osteoporosis by inhibiting the differentiation of osteoblasts from mesenchymal stem cell, inducing apoptosis of osteoblasts and osteocytes, increasing the formation of osteoclasts, and prolonging the lifespan of osteoclasts. The effects of anti-osteoporotic drugs (green lines) such as bisphosphonates, teriparatide, denosumab, and raloxifene are indicated. Bisphosphonates inhibit the activity of osteoclast and induce its apoptosis. Bisphosphonates and the intermittent administration of teriparatide decrease the apoptosis of osteoblasts and osteocytes. Raloxifene, only used for postmenopausal women with glucocorticoid-induced osteoporosis, promotes bone formation by stimulating osteogenesis and suppressing osteoblast apoptosis and indirectly inhibits osteoclastogenesis by decreasing the expression of receptor activator of NF-κB ligand and increasing the expression of receptor activator of NF-κB ligand inhibitor osteoprotegerin. Denosumab inhibits osteoclastogenesis by neutralizing receptor activator of NF-κB ligand. Blue lines indicate the signaling affecting osteoclastogenesis

decades [71,72]. Osteocytes are mechanosensory cells that can sense the microdamage on the bone through their dendritic processes [73] and trigger their apoptosis [73-75]. While osteocytes undergo apoptosis, the neighboring nonapoptotic osteocytes attract osteoclast precursor cells to the microdamage site by releasing IL-6 and soluble IL-6 receptor [76] and secret RANKL to stimulate the osteoclastogenesis [77]. In the regulation of lifespan of cultured osteoblasts and osteocytes, excess glucocorticoids ($\geq 10^{-6}$ M) induced apoptosis [78-80]. This observation is consistent with the *in vivo* experiment showing that excess glucocorticoids increase the apoptosis of osteoblasts and osteocytes [81]. Mechanistically, glucocorticoids could induce the apoptosis of osteoblasts by inhibiting the WNT, TGF-β, and IL-6 signaling [64,65,82].

The excessive glucocorticoids promote the differentiation and survival of osteoclast

The excessive amount of glucocorticoids promote the proliferation and survival of osteoclast precursor cells. Osteoclasts are originated from hematopoietic stem cells which differentiate into osteoclast precursor cells and then fuse to form multinucleated osteoclasts [83]. During osteoclastogenesis, both macrophage colony-stimulating factor (M-CSF) and RANKL play vital roles [84]. M-CSF is required for the cell survival and proliferation of osteoclast precursor cells, whereas RANK is required for the differentiation of osteoclast precursor cells [85-87]. When M-CSF binds to its receptor, colony-stimulating factor 1 receptor (c-Fms), on osteoclast precursor cells, the cell survival and proliferation of osteoclast precursor cells are promoted through the extracellular signal-regulated kinases and Serine/threonine kinase (Akt) signaling pathways [88]. Evidence has shown that glucocorticoids promote the proliferation and survival of osteoclast precursor cells by increasing the expression and half-life of *M-CSF* produced by osteoblast [89,90].

Glucocorticoids also promote osteoclast differentiation. RANKL secreted by both osteocytes and osteoblasts binds to the RANK receptor on osteoclast precursor cells and subsequently activates the mitogen-activated protein kinase, Akt, and nuclear factor of activated T-cells, cytoplasmic 1 signaling, which stimulate the differentiation and fusion of osteoclast precursor cells into multinuclear osteoclasts [91-93]. The activity of RANKL can be neutralized by its decoy receptor OPG secreted by both osteoblasts and osteocytes [94-96]. When *Opg* is expressed in large amounts, it hinders the formation of osteoclasts, resulting in osteopetrosis [94]; conversely, osteoporosis can be observed in *Opg* knockout mice [97,98]. Therefore, the ratio of RANKL/OPG is recognized as an indicator for the trend of osteoclast differentiation. For example, IL-6 enhances osteoclastogenesis by increasing the *Rankl*/*Opg* ratio [99]. Glucocorticoids promote the differentiation of osteoclast precursor cells toward osteoclast by enhancing the expression of *Rankl* from osteoclasts [100,101]. In the other way, glucocorticoids indirectly increase the RANKL activity by reducing the expression of its decoy receptor *Opg*. Glucocorticoids reduce the expression of *Opg* by directly regulating its expression in osteoblasts [100] or indirectly reduce the expression of *Opg* through the suppression of WNT signaling, which promotes the secretion of OPG from osteoblasts and osteocytes [102]. It has also been reported that glucocorticoids stimulate osteoclast formation through the activation of IL-6 signaling in osteoblasts [103], although the detailed mechanism is unclear.

The average lifespan of osteoclasts is around 2 weeks in humans [104]. Glucocorticoids act directly on osteoclasts to suppress their apoptosis and thus prolong the lifespan of osteoclasts [105,106]. On the other hand, glucocorticoids also suppress apoptosis of osteoclast precursor cells by decreasing the expression of *Opg* [107] and increasing the expression of *Rankl* [108]. Although glucocorticoids prolonged osteoclasts' lifespan, it was reported that glucocorticoids reduce osteoclast activity by disrupting M-CSF-stimulated cytoskeletal organization *in vitro* [109].

The impact of therapeutic glucocorticoids on bone matrix

During the process of bone formation, osteoblasts secrete osteoid, the premineralized bone matrix, to prompt bone formation [110] and differentiate into osteocytes embedded in the bone matrix [111]. In osteoid, hydroxyapatite, a complex of calcium and phosphate, is formed within the matrix vesicles that bud from the plasma membrane of osteoblasts [112]. The hydroxyapatite further deposits into the extracellular matrix (ECM) of the bone and interacts with the main fibrous protein, type I Collagen, to form the mineralized collagen essential for maintaining the bone strength [113]. In GIOP patients, glucocorticoids lessen bone mineralization by inhibiting the expression of type I *Collagen* and increasing the expression of interstitial *Collagenase* [114-116].

Osteoblasts also secrete noncollagenous proteins, such as tissue nonspecific alkaline phosphatase (TNAP), osteocalcin (OCN), and osteonectin (ON)/secreted protein acidic and rich in cysteine [117]. These noncollagenous proteins play crucial roles in the bone matrix's mineralization and could be affected by glucocorticoids. TNAP is a membrane-bound enzyme that is localized on the plasma membrane of osteoblasts and the matrix vesicles [118,119]. TNAP can hydrolyze inorganic pyrophosphate (PPi) to phosphate (Pi) for the formation of hydroxyapatite [120]. OCN is a γ-carboxy glutamic acid-containing protein and has a dual function on bone development. In one way, OCN functions as an inhibitor of bone mineralization by binding to calcium, mediating its association with hydroxyapatite; in the other way, OCN and osteopontin enhance the mechanical properties of the bone [121]. Besides, exogenous supplementation of OCN enhances the differentiation of osteoblasts and increases extracellular calcium levels and TNAP activity [122]. As a calcium-binding matricellular protein, ON triggers the release of the calcium ion by binding to both collagen and hydroxyapatite [123], thereby promoting mineralization of the collagen matrix during bone formation. In addition,

ON-null mice have fewer osteoblasts and osteoclasts, leading to a decrease in bone remodeling [124]. As for osteoclast, it also secrets proteolytic enzymes, such as matrix metalloproteinases (MMP) [125] and cathepsins [126,127], for the degradation of the matrix protein of the ECM during bone resorption. The treatment of glucocorticoids negatively impacts the mineralization of bone matrix by reducing the TNAP activity [128], expression of *Ocn* [129-131], and expression of *On* [132] in osteoblasts. Moreover, glucocorticoids increase the expression of *Mmp9*, *Mmp13*, and *Cathepsin K* by osteoclasts and thus promote the bone reabsorption [78,132,133].

Fracture risk assessment for glucocorticoid-induced osteoporosis

For adults ≥40 years of age using glucocorticoids chronically, the fracture risk can be assessed based on BMD and the fragility fracture history. As defined by the World Health Organization in 2008, a BMD T score of <-2.5 standard deviation is considered as osteoporosis. In addition to BMD, the 2017 American College Rheumatology Guideline for the Prevention and Treatment of GIOP recommends using Fracture Risk Assessment Tool (FRAX®, https://www.sheffield.ac.uk/FRAX/) for fracture risk assessment, which is a tool that integrates the information derived from both clinical risk factors and BMD. In the guideline, adults with low FRAX® fracture probability are recommended to take only calcium and Vitamin D, whereas adults with moderate-to-high FRAX® fracture probability (10-year probability of major osteoporotic fracture >10%) are suggested to be treated with additional anti-osteoporosis medication. However, the International Osteoporosis Foundation and the European Calcified Tissue Society suggested that an intervention threshold, instead of the categorization of FRAX® fracture probability, should be determined for clinical practice [134]. Besides, FRAX® fracture probability does not consider the dose of glucocorticoids; therefore, it needs to be adjusted according to the condition of glucocorticoid usage. For example, FRAX® calculations for the 10-year probability of major osteoporotic fracture and hip fracture should be uplifted by 15% and 20%, respectively, when patients take glucocorticoids at doses >7.5 mg/day [135]. In Taiwan, although there is no specific intervention threshold set for GIOP, the 2019 Taiwanese Consensus and Guidelines for the Prevention and Treatment of Adult Osteoporosis suggests using a presumed individual intervention threshold [136]. The presumed individual intervention threshold is defined as the 10-year probability of FRAX®-derived fracture risks for an individual who does not have rheumatoid arthritis, glucocorticoid usage, and other osteoporotic risk factors but has a previous fracture history [136,137]. By comparing it with the adjusted-FRAX® 10-year probability according to the glucocorticoid dosages, the timing of medical interventions could be determined. Besides, a novel hybrid intervention threshold was established to identify high-risk populations of fragility fractures in Taiwan by considering the FRAX®-derived fracture risks probability, BMD, and presumed individual intervention threshold [138]. However, the intervention threshold for GIOP could vary from country to country, depending on the health policy, economic status, and reimbursement issues.

It is to be noted that the FRAX® calculation is not applicable to determine the fracture risk probability for patients <40 years of age. Although young patients quickly regain bone mass when glucocorticoids are discontinued, the use of glucocorticoids at a dose of >7.5 mg/day for 6 months could still lead to a rapid decrease in bone density in hip or vertebrae (a decrease of >10% in one year) [139]. Therefore, both BMD and prior osteoporotic fracture history should be considered when physicians judge medical intervention for individuals <40 years of age.

Treatment options for glucocorticoid-induced osteoporosis

Calcium and Vitamin D supplements

The evaluation indicators of drug therapy include the dosage and duration of glucocorticoid usage, fragility fracture history, BMD, age, and whether the patient is a postmenopausal woman [140]. In general, prophylaxis and treatment should be initiated in patients using glucocorticoids at a daily dose of 5–7.5 mg for >3 months [139]. Patients treated with glucocorticoids have faced systematic calcium loss caused by reduced gastrointestinal absorption and renal tubular reabsorption [141,142]. Therefore, it is suggested that adult patients should take adequate calcium (1000–1200 mg/day) and Vitamin D (600–800 IU/day) supplements to reduce calcium loss from bone and increase calcium absorption in the gastrointestinal tract [139]; for adults >50 years of age, a daily intake of 1200 mg calcium with 800–1000 IU Vitamin D is suggested [136].

Bisphosphonates

Bisphosphonates have a nonhydrolyzable P-C-P structure and are analogs of pyrophosphate. Structurally, the bisphosphonates with a nitrogen-containing side chain on the central carbon exhibit substantial therapeutic effects (e.g., alendronate, risedronate, and zoledronate). Bisphosphonates have a high affinity to hydroxyapatite, and thus they could accumulate on surfaces undergoing active resorption. Upon entry into osteoclasts through endocytosis, nitrogen-containing bisphosphonates inhibit the mevalonate pathway's farnesyl pyrophosphate synthase, thereby blocking protein prenylation, inhibiting the function of osteoclasts [143,144], and inducing osteoclast apoptosis [145,146]. Apart from the major therapeutic effect of bisphosphonates on inhibiting osteoclasts, bisphosphonates can also increase the lifespan of osteoblasts and osteocytes by inhibiting their apoptosis [147]. In the other way, bisphosphonates decrease the expression of the BMP antagonists *Follistatin* and *Dan*, the WNT signaling inhibitors *sFRP-1* and *axin-2* [49], thus facilitating WNT and BMP signaling and eventually increasing osteoblast formation.

Side effects of bisphosphonates may comprise erosive esophagitis, ulcer bleeding, hypocalcemia, renal function decline, osteonecrosis of the jaw, and atypical femoral fracture [148]. The failure of oral bisphosphonate treatment can be defined as GIOP patients who have new fractures after >18 months of oral bisphosphonates or experienced a

significant decrease in BMD (>10% per year) after 1 year of treatment. In such a scenario, follow-up treatment with other osteoporotic drugs, such as denosumab or teriparatide, is suggested [139]. If the failure of oral bisphosphonate treatment is due to poor medical compliance or drug absorption issue caused by gastrointestinal side effects, intravenous bisphosphonates can be considered because of its long dosing interval and infrequent gastrointestinal side effects [149]. Accordingly, a decrease in BMD, new fractures, and other rare side effects, such as osteonecrosis of the jaw and atypical femoral fractures, should be carefully evaluated. For patients who stop using glucocorticoids and have a low risk of fracture, bisphosphonates can be discontinued; however, this is not recommended in patients who have discontinued glucocorticoids but remain at high risk of fracture [139]. It is to be noted that bisphosphonates have a relatively long half-life and tend to be trapped in bones, potentially affecting fetal bones; therefore, they are not recommended for pregnant women [150].

Therapeutic monoclonal antibody

Another commonly used drug in clinical practice is RANKL inhibitor (Denosumab). It is a human monoclonal antibody that binds and neutralizes RANKL, limiting the formation of osteoclasts, thereby inhibiting bone resorption [151]. The clinical trial indicates that GIOP patients take denosumab (60 mg subcutaneously once every six months) has a better therapeutic effect than those take risedronate (5 mg oral per day) in terms of BMD increases in spine and hip after one year of the treatment [152]. The side effects for patients taking denosumab include hypocalcemia, osteonecrosis of the jaw, and a high risk of infection [153,154]. In addition, the incidence of vertebrae compression fracture also increases rapidly after discontinuation of denosumab [155]. Moreover, there may be a risk of fetal teratogenesis when used in pregnant women [156]. An advantage of denosumab is that no dose adjustment is necessary for patients with renal impairment; however, patients with creatinine clearance <30 mL/min or receiving dialysis are at risk for hypocalcemia. A clinical study has shown that denosumab therapy is well tolerated and improves BMD for patients with solid organ transplant, especially in those with renal function impairment or bisphosphonate intolerance [157]. However, a significant decrease of BMD at the lumbar spine and hip was reported when denosumab was discontinued in renal transplant recipients [158]. Therefore, if denosumab treatment is to be discontinued, an alternative anti-osteoporotic therapy should be considered.

Parathyroid hormone N-terminal active fragment

Teriparatide is an active form of parathyroid hormone (PTH) consisting of the N-terminal 34 amino acids. In the clinical survey, teriparatide significantly increases the expression of bone formation markers and bone mass density of GIOP patients [159-161]. Intermittent use of teriparatide facilitates osteoblast production, increases TNAP activity [162], and promotes WNT signaling by reducing WNT signaling inhibitors, such as *Sost*, *Dkk1*, *sFRP-1,* and *axin-2* [49,163-165]. Intermittent administration of teriparatide also inhibits apoptosis of osteoblasts and osteocytes [166,167], thereby promoting bone formation and increasing bone mass. In addition, teriparatide and WNT can synergistically increase the nuclear translocation of β-catenin by PKA-mediated phosphorylation, thus facilitating WNT signaling [165]. In the absence of WNT binding, PTH-PTH1R complex can also bind to WNT coreceptor LRP6 and trigger WNT signaling in osteoblasts [168]. Teriparatide also decreases the expression of BMP antagonists *Follistatin and Dan* to facilitate BMP signaling [49]. Besides, PTH exerts an insulin-like growth factor I-mediated anabolic effect on bone formation [169,170].

However, long-term use of teriparatide may increase *Rankl* expression and inhibit *Opg* expression, causing osteoclast differentiation and increasing the number of osteoclasts, leading to bone resorption and bone loss [171,172]. Furthermore, bone loss and fractures may rapidly occur after teriparatide is discontinued [173]. Accordingly, after teriparatide discontinuation, other osteoporotic drugs should be used. After long-term use of teriparatide, the side effects include a possible cause of osteosarcoma, hypercalcemia, nausea, leg cramps, and dizziness [174].

Selective estrogen receptor modulator

The selective estrogen receptor modulator (SERM), such as raloxifene, lasofoxifene, and bazedoxifene, acts as a tissue-specific agonist and antagonist as it activates estrogen receptors in bone and inhibits estrogen receptors in the uterus and breast [175]. Estrogen facilitates the differentiation of MSCs into osteoblastic lineage [176]. Correspondingly, raloxifene stimulates *Runx2* expression to promote the differentiation and proliferation of osteoblasts and suppresses the production of osteoclasts by inhibiting the expression of *IL-6* [177]. Estrogen inhibits the expression of *Sost* by osteocytes and bolsters WNT signaling, leading to increased osteoblast formation [178,179]. Raloxifene also attenuates the expression of *Sost* and *Dkk1* in mice [180]. In the other way, estrogen could suppress the differentiation of osteoclast precursor cells by decreasing *Rankl* expression and increasing *Opg* expression in osteoblasts and osteocytes [181,182]. Similarly, raloxifene increased the expression of *Opg* and decreased the expression of *Rankl* and *IL-6* in human osteoblastic MG-63 cells [183]. However, different from the effect of estrogen on the regulation of apoptosis [38,69,184], clinical and cell culture studies indicate that raloxifene neither enhances the osteoclast apoptosis [185] nor suppress osteocyte apoptosis [186], except that raloxifene could protect osteoblast from apoptosis induced by sodium nitroprusside [187].

Clinical trials with postmenopausal osteoporotic women indicate that raloxifene [188], lasofoxifene [189], and bazedoxifene [190] are effective for reducing the incidence of vertebral fractures, but not nonvertebral fractures. Among SERMs for the osteoporosis treatment in postmenopausal women, raloxifene is the only SERM approved by the United States Food and Drug Administration (US FDA); the Taiwan FDA approves raloxifene and bazedoxifene. Although the US FDA does not approve the use of raloxifene for GIOP patients, the 2017 American College Rheumatology Guideline for the Prevention and Treatment of GIOP suggests that raloxifene could be used to treat postmenopausal women who have

GIOP but fail to respond to regular GIOP treatment or have specific therapeutic contraindications [139]. It is to be noted that women receiving raloxifene might have an increased risk of venous thromboembolism [191].

Treatment of glucocorticoid-induced osteoporosis in pregnant women and children

Because of the lack of comprehensive medication safety assessments for osteoporotic drugs used in pregnant women, there is no treatment recommendation for pregnant GIOP patients. According to the 2017 American College Rheumatology guidelines, oral bisphosphonates are recommended only when female GIOP patients are not planning to become pregnant and have moderate to high risk of fracture; otherwise, only calcium tablets and vitamin D should be used. However, when the female GIOP patients experience side effects from oral bisphosphonates, teriparatide is recommended. Because of safety concerns, denosumab and intravenous injection of high-potency bisphosphonates are only applicable to the female GIOP patients having a high risk of fracture and avoiding pregnancy when other anti-osteoporotic drugs are not applicable [139].

Glucocorticoids are extensively used in children with various indications because of their significant anti-inflammatory and immunomodulatory activity. A study conducted in the United Kingdom found that 1.2% of children received at least one kind of oral glucocorticoid within a year to treat asthma attacks. Asthma is a chronic, obstructive, and inflammatory lung disease requiring long-term treatment with glucocorticoids adjusted according to each child's response to treatment [192]. Other chronic inflammatory diseases in children requiring long-term treatment with glucocorticoid for >3 months include juvenile idiopathic arthritis, systemic lupus erythematosus, juvenile dermatomyositis, Crohn's disease, and nephrotic syndrome. The glucocorticoids used to control these inflammatory diseases have an additive effect on reducing bone formation and severely compromising children's bone health [193].

An epidemiologic study conducted on the British population (including those aged 4–17 years) showed that oral glucocorticoids used for >4 cycles per year significantly increased fracture risk, with humerus fracture being the most common [194]. Therefore, the treatment of osteoporosis in children (between 4 and 17 years of age) who use glucocorticoids chronically requires a multifaceted approach: (1) Nutritional intake should be actively tracked to prevent obesity and ensure adequate intake of calcium (1000 mg/day), Vitamin D (at least 600 IU/day and exposure to sunlight for approximately 20 min/day), and protein. Furthermore, track the serum concentration of 1, 25-dihydroxyvitamin D every 3–6 months to determine whether the intake dose needs to be adjusted. (2) Regularly perform supervised physical exercises. In addition to controlling ideal body weight, it is also beneficial to maintaining bone and muscle strength. (3) For spontaneous fractures, especially vertebrae fractures (confirmed by pain or height loss), regular radiological examinations are required to rule out the possibility of occult fractures. For patients who have suffered GIOP fractures and continue to use glucocorticoids for >3 months (0.1 mg/kg/day), medical intervention is required [139].

Conclusion

GIOP is the most common type of secondary osteoporosis. It often occurs in patients who used glucocorticoids for a long time, such as those with autoimmune diseases, allergic diseases (e.g., asthma and atopic dermatitis), or organ transplantation. It is an iatrogenic disease in which osteogenesis and osteoclastogenesis are out of balance. Excess glucocorticoids cause rapid bone loss by downregulating bone formation and upregulating bone resorption during the 1st year of glucocorticoid treatment. In addition to direct effects on bone cells, such as osteoblasts, osteoclasts, and osteocytes, glucocorticoids also indirectly cause calcium loss, hypocalcemia, and secondary hyperparathyroidism. Therefore, the dosage and duration of treatment with glucocorticoids should be minimized. Moreover, nonpharmacological treatments, such as appropriate nutrition and exercise, should be combined with pharmacological treatments. For GIOP patients at high risk of fracture, medical intervention is recommended. In the future, more definitive safety studies have to be conducted for the medication of pregnant women and children with GIOP. Due to the limited choices and side effects of the drugs used for GIOP, it is eager to invent more effective and safer therapeutic drugs to meet the best interest of GIOP patients and society.

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Conflicts of interest

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