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PERSPECTIVES

New Insights into the Biology of Glucocorticoid-Induced Osteoporosis

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Abstract

Glucocorticoid (GC) use results in rapid bone loss and elevated fracture risk. The excess bone fragility from GC treatment is multi-factorial. GCs alter calcium and phosphorus metabolism, which can result in elevation of parathyroid hormone (PTH) and early stimulation of osteoclast activity and bone remodeling, followed by a delayed but sustained reduction in osteogenesis, osteoblast activity and osteocyte metabolism. The changes in bone cell viability with GCs results in reduction of localized and whole bone strength. New data reveal that GC use may influence mineral metabolism through FGF23. The altered perilacunar mineralization around GC-treated osteocytes may be secondary to increased FGF23 production. In addition, low doses of GCs can induce self-preservation of osteocytes through the molecular mechanisms of autophagy, while higher doses of GCs induce osteocyte apoptosis. Osteocytic autophagy may allow for cell survival and then, with withdrawal of GCs, for the repair of lost bone tissue. Currently, both anti-resorptive and anabolic agents are prescribed to prevent or treat GC-induced bone loss. Additional studies are needed to further explore whether current treatments used for GC-induced bone loss alter osteocyte metabolism and how this influences localized and whole bone strength. *IBMS BoneKEy*. 2011 May;8(5):229-236.
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Introduction – The Clinical Importance of Glucocorticoid-Induced Bone Loss

Glucocorticoids (GCs) are frequently used in clinical medicine to treat non-infectious inflammatory diseases. However, GC use results in rapid trabecular bone loss and a high incident fracture risk. Epidemiologic studies show 50% of rheumatoid arthritis patients treated with chronic GCs will suffer an osteoporotic fracture; baseline data from randomized clinical trials show a prevalence of vertebral fracture of 30% (1-6). Other studies show that both old and young, men and women, and all ethnic groups studied lose bone mass with GC treatment, making this an important public health problem (7). Because patients treated with GCs may require treatment for a long period of time, thereby increasing their risk of fractures, there is a medical need to understand the biology of GC-induced bone loss so that clinicians can effectively prevent and treat this disease. The observational data from GC clinical studies shows that the initiation of GC treatment is associated with a change in bone metabolism, which in turn leads to a rapid reduction in bone mass at sites rich in trabecular bone, *e.g.*, the vertebra and femur, with incident vertebral fracture risk elevated within 1 year of initiating GC treatment (8-10). Nevertheless, the loss of trabecular mass and architecture do not entirely explain the increase in fracture risk, as individuals treated with GCs frequently experience fractures at higher BMDs than women with postmenopausal osteoporosis (9).

The Biology of GC-Induced Bone Loss

GC treatment results in changes in bone remodeling (8;11). Observations of surface and biochemically-based turnover in clinical studies of glucocorticoid-induced osteoporosis show a reduction in trabecular

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bone volume, thickness and bone formation (8;12-14). The influence of GCs on bone resorption was thought to be indirect and related in part to reduced calcium absorption and increased renal calcium excretion (15). However, recent studies have found that GCs act directly on osteoclasts to decrease the apoptosis of mature osteoclasts (16). Kim *et al*. found that GCs *in vitro* inhibited the proliferation of osteoclasts from bone marrow macrophages (BMMs) in a dose-dependent manner. In addition, higher GC doses had no effect on osteoclast maturation but inhibited osteoclasts from reorganizing their cytoskeleton (17). Therefore, GC excess results in an increase in osteoclast number, but in an apparent inhibition of function with impaired spreading and degradation of mineralized matrix (17).

GCs also alter osteoblast and osteocyte function, which contributes to GC-induced osteoporosis (15). GCs directly inhibit cellular proliferation and differentiation of osteoblast lineage cells (18), reduce osteoblast maturation and activity (11), and also induce osteoblast and osteocyte apoptosis *in vivo* (19). The suppression of osteoblastic function by GCs is reported to be associated with alteration of the Wnt signaling pathway (20), a critical pathway for osteoblastogenesis (21;22). GCs enhance Dickkopf 1 (Dkk1) expression (23), one of the Wnt antagonists that prevent soluble Wnt proteins from binding to their receptor complex (24). GCs maintain levels of glycogen synthase kinase-3β (GSK-3β) (25), a key kinase that phosphorylates β-catenin, thereby preventing the translocation of β-catenin into the nucleus and the initiation of transcription in favor of osteoblastogenesis. GCs may also enhance bone marrow stromal cell development towards the adipocyte lineage rather than towards the osteoblast lineage (22;26). Moreover, the loss of osteocytes by GC-induced apoptosis (27) may disrupt the osteocyte-canalicular network, resulting in a failure to direct bone remodeling at the trabecular surface. GC-induced changes in osteocyte function also result in a weakening of the localized material properties around osteocytes as well as in decreased whole bone strength (28).

Mineral Metabolism and Osteocytes

GC treatment is known to alter calcium metabolism. Treatment with GCs reduces the gastrointestinal absorption of calcium and increases urinary excretion of calcium, which leads to a calcium deficit (15;29;30). Over time this calcium deficit and low serum ionized calcium levels can stimulate parathyroid hormone (PTH) release; PTH then catalyzes 1-α-hydroxylase enzyme production in the kidney, which in turn increases 1,25$(OH)_2$ vitamin D levels, and this is followed by gastrointestinal absorption of both calcium and phosphorus. If the calcium deficit continues, gastrointestinal absorption of these minerals continues, resulting in elevation of serum phosphorus that then stimulates the production of fibroblast growth factor 23 (FGF23) by osteocytes in an attempt to lower serum phosphorus. FGF23 is a hormonal factor that is produced primarily by osteocytes and reduces serum phosphorus and 1,25$(OH)_2$ vitamin D levels by acting on the kidney through FGF receptors and Klotho (31-33). The production and circulating levels of FGF23 appear to be tightly regulated but the mechanisms responsible are still under investigation.

The association between FGF23, osteocytes and mineralization has recently been explored (34). FGF23 serves as a phosphaturic factor synthesized by osteocytes and inhibits 1,25$(OH)_2$ vitamin D production by the kidney to maintain the balance between phosphate homeostasis and skeletal mineralization (35). A recent *in vitro* study demonstrated that overexpression of FGF23 suppressed osteoblast differentiation and matrix mineralization (36). Another study evaluated the proteins associated with osteocytes and bone mineralization and found that FGF23 co-localized to the secondary spongiosa of trabecular bone and areas of cortical bone where the osteocyte lacunar system was mature, suggesting that FGF23 produced by osteocytes would then be part of the bone-

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renal axis that is central to proper mineral metabolism (37;38). Elevated levels of serum FGF23 have been found in individuals with autosomal hypophosphatemic rickets with mutations in *DMP-1* (dentin matrix protein-1) and other forms of rickets and chronic kidney disease exhibit elevated levels of FGF23 despite normal calciuria (39;40). In contrast, mice with deletion of *Klotho* developed elevated DMP-1, hyperphosphatemia and low FGF23 levels (41). Also, overexpression of FGF23 in primary rat calvaria cell cultures suppressed matrix mineralization (36). In one pilot study, increased FGF23 expression in ovine callus was associated with delayed fracture healing (42). Therefore, based on these initial reports and the preliminary data, we examined FGF23 expression in GC-treated mice. It appears that changes in the production and local concentration of this phosphaturic factor by the osteocyte may result in a reduction in osteocyte-driven mineral metabolism, thereby compromising local bone strength (43-45). In GC-treated mice, we have observed a dose-dependent increase in serum FGF23, with a decrease in serum phosphorus and $1,25(OH)_2$ vitamin D, suggesting that GC use may influence mineral metabolism through FGF23. The altered perilacunar mineralization around GC-treated osteocytes may be secondary to increased FGF23 production.

Osteocyte Autophagy Induced by GCs

The autophagy pathway is one of the most important biologic processes that enable cells to survive stress and starvation and help to maintain cellular homeostasis by degrading damaged organelles (46-49). Autophagy is defined by the formation of autophagosomes, also known as autophagic vacuoles, which are lined by two membranes with the recruitment of LC3-phosphatidylethanolamine conjugate (LC3-II) to the autophagosomal membrane, a characteristic of autophagosomes (50). When autophagosomes fuse with lysosomes and form autolysosomes, degradation occurs and amino acids or other small molecules are delivered to the cytoplasm for energy production or recycling. The time the cells spend under stress might result in extensive recycling of damaged organelles that may lead to cell death (47;51;52). Autophagy can be inhibited by chloroquine (CQ) as it accumulates within autophagosomes and then inhibits fusion with lysosomes, thereby preventing the formation of autolysosomes. This reduction by chloroquine in the final phase of autophagy, which provides a pathway for the breakdown of proteins and removal of metabolic debris from the cell, may augment apoptosis (53-55) or rescue osteocytes from cell death (56).

In collaboration with J. Jiang *et al*., we have found that dexamethasone treatment of an osteocytic cell line increased autophagic activity as detected by several standard approaches based on recently published guidelines that included fluorescent GFP-LC3 dots, MDC fluorescence, LC3 lipidation and electron microscopy imaging in addition to conventional acridine orange staining (57). The enhancement of autophagy was also validated in isolated primary osteocytes treated with dexamethasone and osteocytes in bone from mice chronically treated with prednisolone. We also observed that gene and protein expression for components of matrix proteolysis, including matrix metalloproteinases (MMPs), caspases and cathepsins, was increased in cortical bone following GC treatment (22). Because the interior of a lysosome is strongly acidic, as it releases the contents of its vacuole through autophagic flux into the microenvironment of the osteocyte, it may induce matrix proteolysis, and demineralization of bone around the osteocyte that over time may weaken both localized bone tissue and whole bone strength (28). To begin to elucidate how the osteocyte could be modifying its perilacunar matrix, we performed microarray analysis, RT-PCR and immunohistochemistry on selected genes and found that with GC exposure for either 28 or 56 days, the expression of genes associated with mineralization (*DMP-1*, *Phex*, and *FGF23*) and lysosomes (genes coding for MMPs, cathepsins, and proteinases) was significantly higher

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compared to the placebo-control at day 0. In summary, we determined that GC-induced changes in osteocyte metabolism resulted in an increase in osteocyte lacunar size or "osteocytic osteolysis", perilacunar demineralization, localized reductions in elastic modulus around the osteocyte, and production of proteins that inhibit osteoblast function. Non-apoptotic programmed cell death, such as autophagy, may play a role in the osteocyte's response to GC-induced stress.

Early events (day 0-14)	Late events (day 15-56)	Outcomes
↑ osteoclast bone resorption	↓ osteoblast bone formation	Increased bone loss
↑ PTH, 1,25 vitamin D	↑ osteocyte autophagy, apoptosis	↓ localized bone strength
↓ Ca	↓ osteocyte peri-lacunar mineralization	↓ whole bone strength
↑ P	↓ P ↑ FGF23	

OVERALL INCREASE IN BONE FRAGILITY

Fig. 1. Proposed mechanisms and timeline of events in the GC-induced osteoporosis mouse model.

Conclusion

In summary, GC use is widespread in clinical medicine and causes increased bone fragility and fractures. Effects of GCs on bone quantity and quality are multi-functional. GCs induce a negative calcium balance that leads to elevation in calciotropic hormones, serum calcium and phosphorus followed by elevation in FGF23 production by osteocytes. GCs directly alter osteoblast, osteoclast and osteocyte cell fates and functions (Fig. 1). Nearly 50% of individuals treated chronically with GCs will suffer a major osteoporotic fracture. A better understanding of the role of the osteocyte in GC-induced bone fragility will lead to potential preventive treatments.

Conflict of Interest: None reported.

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