

New understanding of glucocorticoid action in bone cells

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Glucocorticoids (GCs) are useful drugs for the treatment of various diseases, but their use for prolonged periods can cause severe side effects such as osteoporosis. GCs have a direct effect on bone cells, where they can arrest bone formation, in part through the inhibition of osteoblast. On the other hand, GCs potently suppress osteoclast resorptive activity by disrupting its cytoskeleton based on the inhibition of RhoA, Rac and Vav3 in response to macrophage colony-stimulating factor. GCs also interfere with microtubule distribution and stability, which are critical for cytoskeletal organization in osteoclasts. Thus, GCs inhibit microtubule-dependent cytoskeletal organization in osteoclasts, which, in the context of bone remodeling, further dampens bone formation. [BMB reports 2010; 43(8): 524-529]

INTRODUCTION

GCs are potent anti-inflammatory and immunosuppressive drugs. As such, synthetic GCs have been widely used for many decades for the treatment of various disorders such as autoimmune, pulmonary, periodontal, and gastrointestinal disease. Although GCs effectively suppress inflammation, its use is accompanied by bone loss leading to osteoporosis, particularly when applied for long time periods. It has been reported that bone loss occurs with a rapid phase of about 12% within the first year of GC administration, followed by a slow phase of 2-5% annually (1, 2). About 30-50% of patients receiving long-term GC therapy suffer severe fractures (3).

GCs have indirect skeletal effects such as decreased calcium absorption in renal tubes and the intestine as well as suppressed synthesis of sex hormones, all of which theoretically induce secondary hyperparathyroidism. Although these indirect effects are considered as a contributory mechanism in the pathogenesis of GC-induced osteoporosis, accumulating evidence has shown that GC therapy does not develop secondary hyperparathyroidism (4-6). Nonetheless, the direct effects of GCs on bone are clear. Therefore, this review summarizes

the molecular effects of GCs on bone cells, specifically on osteoclasts.

Cellular action of the GC receptor

In general, GCs mediate their biological effects through the interactions of their cognate receptor, the glucocorticoid receptor (GR), a member of the nuclear receptor superfamily (7, 8). The GR has a modular structure consisting of an N-terminal domain (NTD), a DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD) (9, 10). In the absence of ligand, GR resides in the cytoplasm as an inactive complex containing molecular chaperons such as heat shock proteins. Ligand binding results in dissociation of this multi-protein complex along with conformational changes of the GR protein. GR then translocates into the nucleus where it regulates the expression of its target gene through several different pathways (Fig. 1).

GR can activate gene transcription by directly binding to glucocorticoid response elements (GREs) in the promoter region of its target genes. This type of regulation has been shown in various genes such as tyrosine aminotransferase, glucocorticoid-induced leucine zipper and phosphoenolpyruvate carboxylase (11). On the other hand, GR also binds to negative GREs (nGREs), which mediate the DNA binding-dependent repression of target gene expression. For example, pro-opiomelanocortin (12), osteocalcin (13) and with-no-lysine (K) kinase-4 (WNK4) (14) contains nGREs. Alternatively, GR can regulate gene expression via protein-protein interactions. In this case, GR associates with other transcription factors such as NF- κ B, AP-1, T-bet, GATA-3, NFAT and IRF3, all of which are involved in the expression of inflammatory cytokines (15-18). Since GR inhibits these inflammation-associated proteins, GCs are widely used for the treatment of inflammation-related diseases.

Glucocorticoids and osteoblasts

GCs inhibit osteoblasts, bone-forming cells, *in vivo* through the induction of apoptosis, which leads to the suppression of bone formation (2, 19). Consistent with these *in vivo* observations, GCs also promote the apoptosis of osteoblasts and osteocytes *in vitro* via the activation of caspase-3 (20-23). In addition, GCs decrease the number of osteoblasts by inhibiting the pool of cells available for differentiation into osteoblasts

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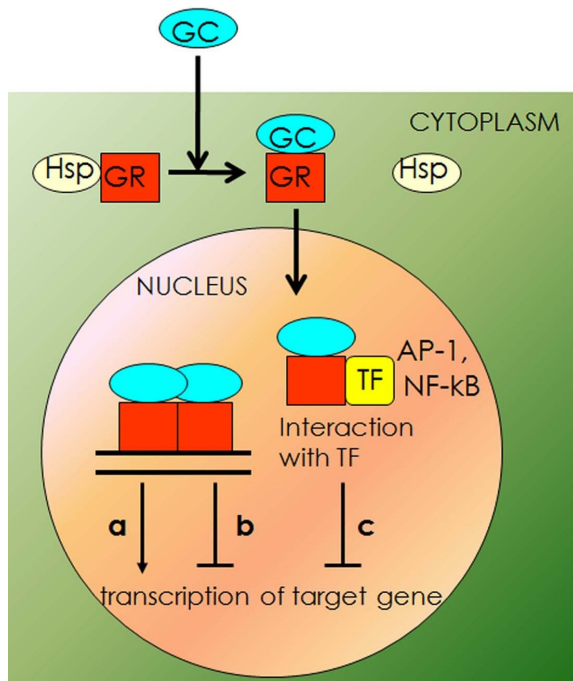


Fig. 1. Action modes of the glucocorticoid receptor. Upon GC binding, GR dissociates from heat shock protein (Hsp) and translocates into the nucleus. The GR as a dimer can interact with GC response elements and activate target gene expression (a), whereas binding of GR to negative GC response elements leads to repression of target gene transcription (b). As a monomer, GR interacts directly with other transcription factors such as AP-1 and NF- κ B and suppresses gene transcription (c). GC, glucocorticoid; GR, glucocorticoid receptor; Hsp, heat shock protein; TF, transcription factor.

(24). GCs further blunt the differentiation of mesenchymal cells into osteoblasts, resulting in a decreased number of mature osteoblasts (25-27). GCs accelerate the shift of bone marrow stromal cells toward adipocyte lineage cells via the enhanced expression of peroxisome proliferator-activated receptor γ , which is an important transcription factor for adipogenesis (26, 28-30).

Although GCs clearly inhibit osteoblast formation *in vivo*, some studies have revealed that exposure to GCs actually promotes the formation of mineralized bone nodules *in vitro* (31-33), indicating that another inhibitory mechanism is active during the suppression of bone formation by GCs *in vivo*.

Glucocorticoids and osteoclasts

Osteoclasts are derived from hematopoietic precursors of the monocyte/macrophage lineage (34). Two cytokines, macrophage colony-stimulating factor (M-CSF) and receptor activator of NF- κ B ligand (RANKL) are essential for osteoclast development.

It has been reported that GCs induce the expression of

RANKL (35) and M-CSF (36) but down-regulate osteoprotegerin (OPG), a decoy receptor of RANKL. Based on these data, GCs would increase the bone-resorption activity of osteoclasts. However, histomorphometric analysis in patients receiving GC therapy indicated a reduction of both bone resorption and bone formation (2, 37, 38). On the other hand, treatment with synthetic GCs, dexamethasone suppresses the formation of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated osteoclasts by downregulating β 3 integrin, which is essential in the modulation of the cytoskeleton (39). Importantly, our recent studies using GR-deficient mice in osteoclast lineage cells have provided a new paradigm for the mechanism of GC-induced osteoporosis (40).

GC effects on osteoclast apoptosis and differentiation

It has been reported that GCs prolong the life span of mature osteoclasts (41, 42). Similarly, in a study with mice lacking the GR, the addition of synthetic GCs promotes the survival of wild-type (WT) osteoclasts but does not affect cells lacking GR (40). On the other hand, GCs do not impact osteoclast differentiation in either WT or GR-deficient KO mice, as determined by the expression of osteoclastogenic markers including TRAP, MMP-9 and Cathepsin K.

GC effects on osteoclast cytoskeleton

Bone resorption is a hallmark of osteoclasts and is initiated by the attachment of mature resorptive cells to the bone surface (34, 43, 44). Upon bone matrix recognition, osteoclasts begin to organize their cytoskeleton, leading to the formation of a unique actin ring or sealing zone. These events are pivotal for the bone resorptive activity of mature osteoclasts. It is known that several cytokines including M-CSF, RANKL, TNF- α and IL-1 α induce actin reorganization in mature osteoclasts (45-47). Although GCs do not impair actin rings of GR-deficient osteoclasts, they specifically inhibit M-CSF-induced actin ring formation in WT osteoclasts, but not RANKL, TNF- α and IL-1 α mediated actin ring formation (40).

The Rho family of small GTPases including Rho and Rac is important for actin cytoskeletal organization and therefore plays a critical role in the bone resorptive activity of mature osteoclasts (48). The expression of constitutively active RhoA accelerates podosome and stress fiber formation, osteoclast motility and bone resorption, whereas a dominant negative form of RhoA and C3 exoenzyme, which specifically inactivates Rho proteins, blocks these events (49). Like Rho, Rac plays an important role in actin ring formation. Rac1-deficient mice show increased trabecular bone volume and trabeculae number due to defective actin cytoskeletal organization (50). Reflecting the inhibitory effect of steroids on actin ring formation, GCs completely arrest M-CSF-induced activity of GTPases (40).

Rho GTPases transit between an activated GTP-bound and inactivated GDP-bound state. This transition is regulated by

GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs). While GAPs stimulate intrinsic GTPase activity, GEFs convert Rho GTPases from their inactive GDP-bound form to their active GTP-bound form (51-53). Vav family proteins including Vav1, Vav2 and Vav3 are examples of GEFs. Among them, Vav3 is critical for cytoskeletal organization in osteoclasts. Vav3-deficient mice have increased bone mass due to impaired bone resorptive activity. Osteoclasts derived from Vav3-null mice show the defect in M-CSF-induced Rac activation *in vitro*, resulting in failed organization of their cytoskeleton (54). Similar to the effects of GCs on Rho and Rac activation, GCs suppress M-CSF-mediated Vav3 activation as well. Thus, GCs arrest organization of osteoclast cytoskeleton by inhibiting M-CSF-induced Vav3 activation and therefore Rac activation (40).

It is known that GCs exert their effects via genomic or non-genomic mechanisms. The non-genomic effects occur within a few seconds to a few minutes and are mediated by membrane-bound GR or by direct interactions with biological membranes (55). Since GC-mediated suppression of Vav3 requires 16 hours, GCs exert their inhibitory effect on organization of osteoclast cytoskeleton through a genomic mechanism.

GC effects on osteoclast microtubule distribution and stability

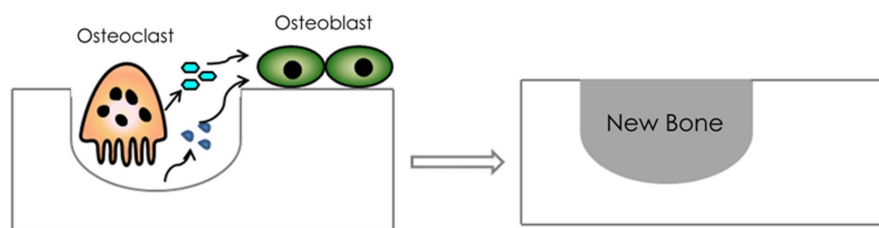
Cytoskeletal organization in osteoclasts requires an intact mi-

cro-tubule network (56, 57). We found that GCs interfere with the distribution of microtubules in mature osteoclasts (Hong *et al.*, unpublished). GC-untreated control cells contain characteristic radial microtubules which are enriched around the actin ring. In contrast, GC-treated osteoclasts possess an irregularly shaped microtubule network that is not concentrated at the periphery of the osteoclast. In addition to intact microtubule distribution, the stability of microtubules is also critical for cytoskeletal organization in mature osteoclasts (56, 57). The level of microtubule acetylation reflects the stability of microtubules. We observed that GC-treated osteoclasts contain very few acetylated microtubules (Hong *et al.*, unpublished). Thus, GCs suppress the stability and distribution of microtubules in osteoclasts and therefore regulate microtubule-dependent organization of the cytoskeleton, which in turn inhibits osteoclast resorptive activity.

GC effects on bone remodeling

The adult skeleton constantly undergoes bone remodeling, an event which replaces old bone with new. Bone remodeling is initiated by osteoclasts that absorb the old bone matrix, followed by movement of osteoblast precursors into the resorption lacuna and the synthesis of new bone matrix. In the context of bone remodeling, a bone formation assay was performed using double tetracycline labeling. While GC-treated WT mice show reduced levels of bone formation and bio-

A. Normal bone remodeling



B. Abnormal bone remodeling in glucocorticoid excess

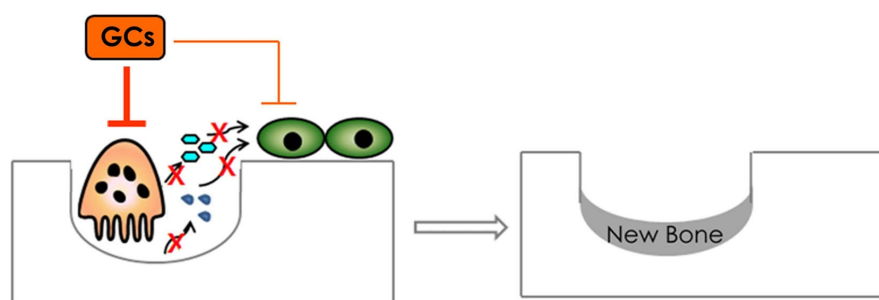


Fig. 2. Effects of GCs on bone remodeling. (A) Normal bone remodeling. Activated osteoclasts resorb old bone matrix. Subsequently, osteoblasts migrate into the resorption lacuna, by factors produced by the osteoclasts or released from the bone. Osteoblasts then synthesize new bone. (B) Abnormal bone remodeling caused by GC excess. GCs directly inhibit osteoblasts. In addition, GCs arrest osteoclast function by suppressing its cytoskeletal organization and microtubule acetylation, which, in terms of bone remodeling, leads to failed recruitment and activation of osteoblasts, further reducing bone formation.

chemical markers including osteocalcin and alkaline phosphatase, GR-deficient mice are protected from GC-mediated suppression of bone formation (40). Thus, GCs cause abnormal bone remodeling by blunting the cytoskeletal organization of osteoclasts, which further suppresses bone formation and ultimately induces osteoporosis.

CONCLUSION

GC-induced bone loss is the most common cause of secondary osteoporosis. GCs impact bone directly. GCs suppress osteoblast cell number, differentiation and function. Furthermore, GCs potently inhibit osteoclast function, which, in the context of bone remodeling, leads to the additional suppression of osteoblast function (Fig. 2). Thus, GCs arrest bone formation by inhibiting osteoclast activation, which is an initial event during bone remodeling. Recently, we have identified target molecules downstream of GCs using microarray analysis. The role of target molecules of GCs in osteoclasts is currently under investigation (Hong *et al.*, unpublished data). These studies will provide insights into the molecular mechanism by which GCs arrest bone resorptive function in osteoclasts.

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