Osteocyte-Mediated Remodeling of the Perilacunar Bone Matrix is Repressed by Glucocorticoids

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Introduction: Despite their central importance as a clinical therapy, glucocorticoids have serious side effects on skeletal health (1). Glucocorticoid treatment causes bone fragility by compromising bone mass and bone quality. Glucocorticoids regulate osteoblast, osteocyte, and osteoclast function and apoptosis (2). Although these cellular mechanisms underlie the glucocorticoid-dependent regulation of bone mass, the mechanisms by which glucocorticoids compromise bone quality remain unclear. Osteocytes play a critical role in the maintenance of bone quality by remodeling the local perilacunar bone matrix (3). In the process of perilacunar remodeling (PLR), osteocytes create an acidic microenvironment and secrete proteases including cathepsin K and matrix metalloproteases (MMP). PLR maintains systemic mineral homeostasis, the canalicular network, and the composition and organization of the mineral and organic bone matrix (3,4). Many questions remain about the mechanisms by which PLR is controlled in bone homeostasis and how it is deregulated in bone disease. For example, the extent to which PLR is a target of glucocorticoids in the deregulation of bone quality remains unclear. Therefore, here we test the hypothesis that glucocorticoids repress the expression of PLR enzymes to impair osteocyte-mediated perilacunar remodeling and the maintenance of canalicular networks and bone matrix integrity.

Methods: To test this hypothesis, we evaluated the effect of the glucocorticoid dexamethasone sodium phosphate on PLR enzyme expression in vitro and in vivo, as well as on histologic outcomes of PLR. RNA was isolated from MLO-Y4 cells, an osteocyte cell line, following a 24h incubation with dexamethasone (1 μm) or vehicle for generation of cDNA and qRT-PCR analysis. As described (5), RNA was isolated from flushed femoral cortical bone dissected from BALB/cJ mice following 14 days of treatment with dexamethasone (0.1 mg/ml) or vehicle by the drinking water. Using defined primer sets (3), the expression of MMP2, 13, and 14 was normalized to the expression of ribosomal protein L19. Contralateral femora from the same mice were processed for frozen sectioning and analyzed using thionin staining and second harmonic generation microscopy to visualize and quantify canalicular and collagen organization, respectively (3). Immunohistochemical analyses used anti-MMP13 (Abcam ab39012). Custom and plug-in NIH ImageJ macros were used for quantitative analyses. Results are representative of N≥3 mice or in vitro biological replicates.

Results: Glucocorticoids rapidly repress MLO-Y4 osteocyte expression of proteases implicated in PLR, including MMP2, MMP13, and MMP14. For example, the expression of MMP13 is repressed 5-fold within 24h of dexamethasone exposure, relative to vehicle-treated cells (N=4, P&lt0.05). To examine the effect of glucocorticoids on PLR enzyme expression in vivo, wild-type mice were treated with
dexamethasone or vehicle for 14 days. Glucocorticoids repressed MMP13 mRNA expression in bone 2-fold (N>5, P<0.05). Immunohistochemical staining for MMP13 protein expression in femoral subchondral trabecular bone revealed that glucocorticoids reduce the intensity and number of MMP13-stained osteocytes. Since glucocorticoids repress the expression of the key PLR enzyme MMP13, we examined the effect of glucocorticoids on PLR outcomes including canalicular and collagen organization. Thionin staining qualitatively showed a glucocorticoid-dependent reduction in canalicular density, which corresponded to a quantitative reduction of canalicular number and area (N>3, p<0.05). PLR-dependent maintenance of collagen organization was also compromised by glucocorticoid treatment. Second harmonic generation microscopy revealed a significant loss of collagen orientation in glucocorticoid treated bone relative to vehicle-treated controls (N=3, p<0.05).

Discussion: Glucocorticoids repress the expression of PLR enzymes and outcomes of osteocyte-mediated perilacunar remodeling. Together with studies that implicate MMP13 and glucocorticoids in the regulation of perilacunar bone matrix composition and organization (3,6), these data suggest that deregulation of PLR may be one mechanism by which glucocorticoid treatment impairs bone quality. Because glucocorticoids also induce osteocyte apoptosis (7), a critical future direction is to assess the extent to which the glucocorticoid-mediated regulation of PLR is downstream or independent of apoptosis. In vitro and in vivo studies are underway to dissect this important regulatory relationship. Either way, understanding the role of PLR in the loss of bone quality following glucocorticoid treatment may implicate PLR enzymes as novel therapeutic targets to prevent or treat bone fragility.

Significance: Dissecting the molecular regulation of osteocyte-mediated perilacunar remodeling, by glucocorticoids and other factors, is essential for understanding the mechanisms that maintain bone quality and those that cause bone fragility in disease.

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