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Glucocorticoid-induced bone fragility:

New insights

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Abstract

Glucocorticoid (GC) use results in rapid bone loss and an elevated risk of fracture. The excess bone fragility from GC treatment is multifactorial. GCs increase bone remodeling through reductions in gonadal hormones, elevations in PTH from negative calcium balance, early stimulation of osteoclast maturation and activity, and delayed, sustained reduction in osteogenesis and osteoblast activity. GCs also alter the metabolism of osteocytes so that increased osteocyte lacunae size, with demineralization around the osteocyte and reduced elastic modulus, is observed in a mouse model of GC-induced bone loss. In summary, GC effects on bone fragility are multifactorial, and additional studies are now under way to clarify how GCs alter osteocyte metabolism and result in reduction in localized bone strength.

Keywords

glucocorticoids; osteoporosis; bone fragility

Glucocorticoids (GCs) are commonly used in clinical medicine to treat inflammatory and autoimmune conditions. While chronic GC use is associated with a number of adverse events, including cataract formation and elevated blood sugar and blood pressure, bone loss and fractures are the most common and severe side effect. Of interest, GC use is associated with rapid bone loss and fragility fractures, which occur often with rather low doses of GCs and with bone mineral density levels that are generally higher than those of postmenopausal women with osteoporotic fractures.[1,2] The observations that GCs increased bone fragility more than we could observe with changes in BMD thus became the focus of our research.

To better define the multiple effects that GCs could have on bone metabolism, we performed a preclinical study using a 5-month-old Swiss-Webster male mouse GC model. Mice were treated with placebo or prednisolone (5 mg 60-day slow-release pellets) for 7–56 days. Our study end points included trabecular bone mass and architecture assessed by microCT scan, bone turnover assessed by biochemical markers (CTX-1, and osteocalcin) and surface-based

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Conflicts of interest

The authors declare no conflicts of interest.

histomorphometric analysis, and microarray studies followed by RT-PCR for gene expression from bone collected from the GC-treated mice.[3,4]

We found that GC treatment resulted in a rapid loss of trabecular bone (nearly 20%) by day 28, whereas very little loss was observed between days 28–56. This loss was accompanied at day 7, and continued to day 28, with an increase in osteoclastogenic gene expression (Csf-1, C-fms, Itgb3, Adams 8, Trem-2, Oscar, Ply, Nfatc1). Also, the biochemical marker of osteoclast activity, CTX-1, increased by day 7 and remained significantly higher than the control values through day 28. Osteoclast surface was increased by about onefold at day 28 and started to decline to thereafter, but it was still significantly higher than the placebo control at day 56.

Osteoblastogenesis was also affected by the GC treatment. However, the changes in bone formation were delayed and were not significant until after 28 days of GC exposure. Gene expression for Wnt signaling inhibitors (Dkk-1, Sost, Wif1) had increased expression from day 28 to day 56. The levels of the biochemical marker of osteoblast activity, osteocalcin, were significantly lower in the GC-treated mice compared to the control levels at day 28 (at least 75% below the control levels) and remained low through day 56. Surface-based bone turnover in the GC-treated mice was also significantly lower than the control animals. In fact, there was very little double-label observed in the GC-treated mice. Since Wnt/beta-catenin signaling is known to be involved in osteoblastogenesis, it appeared that GC treatment reduced osteogenesis through the increased expression of Wnt signaling inhibitors.

Our initial evaluation of bone metabolism in the presence of GCs using a small animal model revealed rapid trabecular bone loss, and this was associated with early accelerated osteoclast maturation, with its accompanying increased bone resorption and a delayed but sustained reduction in osteoblast maturation and activity. However, the changes in bone metabolism, while significant, probably were not sufficiently robust to fully explain why patients treated with GCs have greater bone fragility than postmenopausal women with similar BMD.

To try to evaluate other aspects of bone quality we used nanoindentation with an adapted scanning probe microscope to observe the trabecular bone surface. This application provided an elastic modulus surface map across the trabeculae. Compared to the control mice, in which the surface-based elastic modulus was relatively uniform and reduced modulus was present for osteocyte lacunae, the GC-treated mice showed a very heterogeneous trabeculae elastic modulus. For example, there were areas around the perimeter in which the elastic modulus was low, although the most striking finding was that there were areas around the osteocyte lacunae in which there was a reduced modulus and the osteocyte lacunae were larger in the GC-treated mice compared to the control mice.[3] We then performed Raman microscopy of the trabecular specimen and found reduced mineral in the areas that corresponded to the reduced elastic modulus by SPM. By using an X-ray tomography microscope (XTM), we were able to obtain mineral distribution at a resolution of 2μm. We found that GC treatment lowered the average mineral levels and shifted the mineralization distribution to lower mineral concentration.[3] Crystal orientation was disorganized compared to that of the placebo.[3] Our findings suggested that apart from moderate decrease in bone

mass, GC treatments induce deterioration in bone quality such that whole bone strength decreases.

The observation that the size of lacunae in osteocytes can increase is not novel: It was reported more than 40 years ago in iliac crest bone biopsies from patients with secondary hypoparathyroidism and other diseases involved with mineralization.[5] Interestingly, in osteocytes we found that for genes involved with bone matrix mineralization (Dmp-1, Phex, Mmp13, Spp1) the gene expression as well as the levels for an inhibitor of osteoblastogenesis (sclerostin) were increased at day 28 and day 56 in GC-treated animals compared to the control mice.[4,6] While we do not yet know how inhibition of mineralization or demineralization is initiated around the osteocyte's lacuna, resulting in its enlargement, this may be associated with reduced localized material changes in the bone. For example, osteocytes are responsible for mechanosensation of loading and, through their extensive canalicular network, can send signals to the bone surface to influence bone remodeling.[7] The size of the osteocyte lacunae may be an important factor for how the cell dissipates the loads it receives. Large osteocyte lacunae are less able to dissipate loads and may crack around the surface and weaken the localized bone, therefore contributing to whole-bone failure.[8] Indeed, elderly individuals are reported to have larger osteocyte lacunae and a greater number of micro-cracks around them. If this localized change around osteocytes in GC-treated mice results in localized bone strength and contributes to bone fragility in GC-treated patients, then this mechanism requires further study.

Our research raised the question of whether osteocytes might contribute to bone mineral homeostasis. Osteocytes, while buried in the bone matrix, are connected to one another and to the bone surface. In addition, osteocyte lacunae have been reported to change size in clinical situations in which there is calcium deficiency, including that with lactation, glucocorticoid treatment, hypophosphatemic rickets, and prolonged estrogen deficiency. Other investigators have stated that the trabecular and endocortical bone surface are not sufficient for osteoclasts to maintain serum calcium balance in those clinical states in which calcium is in high demand. There is evidence that osteocytes express metalloproteinase enzymes and secrete them in the local environment, which may then become a source of both calcium and phosphorus for the body. It is possible that the size of the osteocyte lacunae may change often, but this has not come to clinical attention because after the calcium demand is met, the size of the lacuna returns to normal. However, in situations in which the osteocyte lacunae do not return to normal when the calcium deficiency is prolonged, as in chronic GC treatment, the cell may undergo autophagy and apoptosis. The large osteocyte lacunae and demineralized area around the osteocyte may over time weaken the localized area of bone, resulting in bone failure.

In this study we found that GC treatment of male mice resulted in a rapid increase in osteoclastogenesis, delayed but prolonged osteoblastogenesis, and reduced trabecular bone mass. In addition, GC treatment reduced the gene expression of proteins secreted by the osteocyte that augment mineralization. We observed increased osteocyte lacunae size, reduced mineralization and elastic modulus around the osteocyte lacunae with fewer than 5% apoptotic osteocytes present. We speculate that the increased bone fragility observed with GC treatment results from a combination of reduced trabecular bone mass and localized

changes within the bone around the osteocyte that reduce the localized material properties of the bone. Bone-active agents that reduce bone turnover, or increase bone formation, are effective in both the prevention and treatment of the increased bone fragility that results from GC treatment. Further research is warranted to better define the factors that stimulate osteocyte demineralization in the presence of GCs and other diseases associated with calcium deficits and how to prevent and reverse this process.

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