Glucocorticoids and the osteoclast

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ABSTRACT

Glucocorticoid-induced osteoporosis uniformly represents suppression of bone formation. The steroid's effects on osteoclasts are, however, controversial. While glucocorticoid administration to patients with inflammatory diseases accelerates bone resorption, osteoclast function falls below normal with prolonged treatment. Thus, administration of anti-resorptive agents, such as bisphosphonates, is justified during early glucocorticoid therapy, but further suppression of osteoclasts, by these drugs, in chronically treated patients will dampen bone remodelling and may compromise skeletal quality, predisposing to fracture.

The quantity and structure of the skeleton are dictated by bone-degrading osteoclasts and osteogenic osteoblasts. All forms of osteoporosis reflect an imbalance of the activities of these cells in which bone resorption supersedes formation.

Glucocorticoids are the most common cause of secondary osteoporosis and suppressed osteoblastic bone formation is a major contributor to such bone loss (1-4). The effect of glucocorticoids on osteoclasts is, however, controversial. Suggesting glucocorticoids stimulate the resorptive process, patients with inflammatory disorders, such as rheumatoid arthritis, experience increased bone degradation as documented by enhanced circulating CTx, during the first three months of therapy (5). These observations, suggesting that glucocorticoids universally stimulate osteoclasts are challenged, however, a study of normal volunteers who received the steroids for 5 weeks (6). In contrast to rheumatoid patients, these non-inflamed individuals experienced no increase in bone resorption which actually decreased in tandem with bone formation.

To explore this conundrum we administered parathyroid hormone, in the presence and absence of dexamethasone, to wild type mice (7). As expected, parathyroid hormone markedly increased circulating markers of bone resorption in the absence of the glucocorticoid receptor. Conjointly administered dexamethasone, however, completely ablated the resorption-enhancing properties of parathyroid hormone. The fact that the steroid did not curtail the osteoclastogenic properties of the hormone indicates its anti-resorptive effects reflect blunted activity of the individual mature osteoclast and not its arrested differentiation.

Cytoskeletal dysorganisation is among the most common causes of the inability mature osteoclasts to resorb bone (8). The osteoclast cytoskeleton is unique as it forms actin rings or "sealing zones" which isolate the resorptive microenvironment, at the cell-bone interface, from the general extracellular space. This gasket-like structure, which appears only when the osteoclast is juxtaposed to mineralised tissue, enables the cell to generate an acidic micoenvironment and accumulate bone degrading enzymes, such as cathepsin K, in the resorptive space. As actin ring formation is a hallmark of cytoskeletal organisation in the osteoclast, we asked if its formation is impacted by glucocorticoids. Thus, we generated osteoclasts on bone and visualised the actin rings by phalloidin staining which delineates fibrillar actin (7). These structures are abundant in the absence of dexamethasone but exposure to the steroid virtually eliminates them.

The osteoclast cytoskeleton is organised by a canonical signalling pathway emanating from the $\alpha v\beta 3$ integrin (9). In this circumstance the osteoclastogenic cytokines, RANKL and M-CSF, which also activate the mature osteoclast, partner with the integrin to activate a $\beta 3$ associated complex consisting of c-Src, Syk, Dap12, Slp76, Vav3 and ultimately, Rac. Deletion of any of these signalling components yields osteoclasts which fail to spread and are deficient in actin ring formation.

Competing interests: none declared.

As Rac is presently the most distal, established member of this complex we asked if its activation, manifest by GTP association, is inhibited by glucocorticoids (7)? Whereas M-CSF promotes transition of Rac from its GDP- to GTP- bound state, this process is completely arrested by dexamethasone. Thus, glucocorticoids inhibit bone resorption by preventing activation of a canonical cytoskeleton organising signalling pathway.

We next attempted to identify a master regulator of the osteoclast cytoskeleton targeted by glucocorticoids. To this end, we performed microarray analysis and discovered that osteoclast expression of calpain-6 is reduced approximately 14 fold by dexamethasone (10). Importantly, siRNA knockdown of calpain-6 produces an osteoclast phenotype mirroring that of dexamethasone exposure. Calpain-6 is a member of the calpain superfamily but is atypical as it lacks proteolytic activity. Its suppression by dexamethasone suggested that diminished calpain-6 plays a central role in the effects of glucocorticoids on the osteoclast cytoskeleton. We established such is the case by overexpressing calpain-6 in WT osteoclasts. Whereas dexamethasone dramatically disrupted the façade of these cells, the effects of the steroid were completely normalised in the transductants. To further explore this issue we turned to the β3 integrin whose occupancy and partnership with RANKL and M-CSF is the proximal event promoting osteoclast cytoskeletal organisation. In fact, calpain-6 siRNA suppresses β3 integrin expression, an event mirrored by exposure to dexamethasone. Thus we propose that the disruption of the osteoclast cytoskeleton, by glucocorticoids, reflects suppressed expression of calpain-6. This paucity of calpain-6, in turn, reduces β3 integrin expression leading to the characteristic poorly spread phenotype of osteoclasts with cytoskeletal dysorganisation.

These data indicate that glucocorticoids suppress both osteoblast and osteoclast function. They also call into question, however, the mechanisms responsible for the fact that bone loss following glucocorticoid treatment is most robust within the first 3 to 6 months. While speculative, this initial rapid loss of bone may reflect persistence of the prior effects of inflammatory cytokines, such as TNF-α and IL-1 as well as the osteoclastic cytokine, RANKL. This hypothesis holds that early in the course of therapy, prior and persistent effects of inflammatory cytokines and RANKL, override glucocorticoid-inhibition of osteoclast function. This hypothesis, if true, would explain the increased resorption attending decreased formation of early glucocorticoid exposure. In fact, early glucocorticoid treatment of patients with multiple sclerosis results in an immediate reduction of bone formation and a transient increase in resorption (11). On the other hand, late in the course of glucocorticoid therapy, when bone loss has attenuated relative to the first 3 to 6 months, the steroids suppress both formation and resorption. Bone quality is a poorly appreciated concept which focuses on the relationship of the abundance of bone and its resistance to fracture. When bone quality is compromised, fracture resistance at any given bone mineral density, is diminished (12). Interestingly glucocorticoids not only reduce bone mass but also compromise quality (13).

The pragmatic implications of poor bone quality relate to bone remodeling. Bone remodeling is an ever occurring event in which the activities of osteoclasts and osteoblasts are coupled. Importantly, the principal role of bone remodeling is likely maintenance of its quality by removing effete skeletal tissue and replacing it with new (12). As glucocorticoids suppress both arms of the remodeling process, its reduction of bone turnover is a likely cause of the poor bone quality experienced by patients receiving these drugs. These observations call to question the current recommendations of most international rheumatological organisations that all patients receiving glucocorticoids be treated with bisphosphonates which are also potent inhibitors of bone remodeling. Not only do anti-resorptive agents, such as denosumab and alendronate, suppress bone resorption, but they also diminish bone formation (14). Thus the use of these agents, in the context of glucocorticoid therapy, would further attenuate bone remodeling thereby promoting poor bone quality. Bisphosphonates, therefore, are probably appropriate agents to inhibit the robust bone loss occurring early in glucocorticoid therapy when resorption is accelerated. However, their later use, when resorption is suppressed by the steroid, is questionable as they will further dampen remodeling and may additionally compromise bone quality. The increasing evidence that bisphosphonate-suppressed remodeling likely predisposes a subset of patients to atypical, poorly healing fractures accentuates this concern. Thus, while optimal strategies for treating chronic glucocorticoid-induced bone loss are not presently established, the use of anti-resorptive agents beyond the first two years of therapy should be approached with caution.

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