

Mini Review

Roles of osteoblasts, osteoclasts, T cells and cytokines in glucocorticoid-induced osteoporosis

Toru Yago*, Yuki Nanke, Manabu Kawamoto, Takefumi Furuya, Tsuyoshi Kobashigawa, Naomi Ichikawa, Naoyuki Kamatani, and Shigeru Kotake

Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan

Steroid-induced osteoporosis is the most common form of secondary osteoporosis. The mechanisms of glucocorticoid (GC)-induced osteoporosis may be divided into indirect and direct effects. A putative indirect mechanism of GC-induced osteoporosis is thought to be secondary hyperparathyroidism or estrogen deficiency. Recent studies, however, suggest that the direct action of GC on bone metabolism is more important. GC stimulates the expression of the receptor activator of NF- κ B ligand (RANKL) and inhibits osteoprotegerin (OPG) expression in a dose-dependent manner in human osteoblasts. The resultant increase in the RANKL:OPG ratio results in the induction of osteoclastogenesis. GC predominantly inhibits human osteoblast proliferation and enhances the differentiation of human-pre-osteoblasts. GC also acts directly on human osteoblasts to up-regulate the expression of M-CSF which is an essential cytokine for the survival of osteoclast precursors. The addition of GC to the culture of human peripheral blood mononuclear cells (PBMC) results in a marked increase in the formation of osteoclasts and an increase in lacunar resorption. Moreover, GC has been shown to inhibit apoptosis in mouse osteoclasts. In addition, the direct effect of dexamethasone (Dex) upon human osteoclastogenesis from PBMC is mediated via the balance between RANKL and IFN- γ in activated CD4 T cells. Dex induced human osteoclastogenesis without adding osteoblasts or soluble RANKL. Dex dose-dependently increased the ratio of RANKL-positive cells to IFN- γ positive cells (RANKL:IFN- γ ratio) only by reducing IFN- γ -positive cells, suggesting that Dex stimulates osteoclast differentiation by elevating the RANKL:IFN- γ ratio in CD4⁺ T cells. The balance between RANKL and OPG (RANKL:OPG) or RANKL and IFN- γ (RANKL:IFN- γ) on osteoblasts and T cells may have an important role in GC-induced osteoclastogenesis. Thus, therapeutic modulation of the expression of RANKL, OPG and IFN- γ by osteoblasts and T cells represents a novel strategy to prevent GC-induced osteoporosis.

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* Correspondence should be addressed to:

Toru Yago, Institute of Rheumatology, Tokyo Women's Medical University, 10-22 Kawada-cho, Shinjuku-ku, Tokyo 162-0054, Japan. Phone: +81-3-5269-1725, Fax: +81-3-5269-1726, e-mail: toruyago@ior.twmu.ac.jp

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Introduction

Long-term administration of glucocorticoid (GC) causes osteoporosis characterized by rapid and severe bone loss together with slow and prolonged bone disruption. The mechanisms of GC-induced osteoporosis have been thought to result from indirect or direct effects of GC. An indirect mechanism of GC-induced osteoporosis is prolonged secondary hyperparathyroidism¹⁾ or estrogen deficiency²⁾. More recently, however, it has been shown that elevated levels of parathyroid hormone or subnormal vitamin D metabolite concentrations are not typical of patients receiving GC therapy. In addition, there is no direct evidence that GC-induced hypogonadism is responsible for enhanced bone resorption. Thus, these findings suggest the existence of additional mechanisms for GC-induced bone loss³⁾ and suggest that the direct action of GC is more important. Bone mass is maintained as a consequence of the balanced activities of bone-forming osteoblasts and bone-resorbing osteoclasts. Key cytokines that regulate osteoclastogenesis are RANKL, which stimulates osteoclast differentiation, activity and survival, and OPG, which inhibits osteoclastogenesis; therefore, the balance between RANKL and OPG expression has an important role in the regulation of osteoclastic bone resorption. This article focuses on the direct effects of GC on human osteoblasts, osteoclasts and T cells.

Roles of GC on osteoblasts

GC acts directly on human osteoblasts and differentially regulates the expression of RANKL and OPG with up-regulation of RANKL and down-regulation of OPG⁴⁻⁵⁾. Similarly, a recent study demonstrated that Dex inhibited OPG mRNA expression, stimulated RANKL mRNA expression and increased the RANKL:OPG ratio in a dose-dependent manner in human primary osteoblasts⁶⁾. Hofbauer et al⁴⁾ also reported that GC acts directly on human osteoblasts to up-regulate the expression of M-CSF, which is an essential cytokine for the survival of osteoclast precursors. The increased M-CSF level may contribute to GC-induced bone resorption. Decreased circulating OPG levels have also been found in the peripheral blood of patients receiving GC treatment⁷⁾. Moreover, GC predominantly inhibits human osteoblast proliferation⁸⁾ and enhances the differentiation of human-pre-osteoblasts⁹⁾. More recently, it has been reported that Dex-suppressed bone formation is mediated through osteoclasts¹⁰⁾.

Roles of GC on osteoclastogenesis

The addition of 10^{-8} M Dex between days 7 and 14 of the 21-day culture of human PBMC in the presence of M-CSF and soluble-RANKL results in a marked increase in the formation of

osteoclasts and an increase in lacunar resorption. On the other hand, the addition of Dex to PBMC cultures after 14 days reduces the extent of lacunar resorption compared to control cultures¹¹⁾.

In relation to the findings described above, several studies suggesting a direct effect of GC on the life span of osteoclasts have been reported. Studies using mouse and rat osteoclasts have reported contradictory results with GC inhibiting apoptosis of mouse osteoclasts¹²⁾ or enhancing apoptosis in rat osteoclasts¹³⁾. Moreover, in a human cell culture system, treatment with Dex did not affect the life span of mature human osteoclasts⁶⁾. These differences in the effect of GC on osteoclast life span may thus be species dependent. In human studies, several reports have shown an increase in osteoclast numbers as well as an increase in osteoclastic activity in the bones of patients receiving GC treatment¹⁴⁾.

New pathological findings of GC-induced osteoporosis *in vitro*: RANKL and IFN- γ expression of T cells

We have demonstrated that IFN- γ -positive human T cells also express RANKL and induce human osteoclast formation by excess RANKL over IFN- γ , suggesting that human osteoclastogenesis by activated T cells is dependent upon the balance between the inductive effects of RANKL and the inhibitory effects of IFN- γ ¹⁵⁾. We recently reported that the direct effect of Dex on human osteoclastogenesis from PBMC is secondary to the balance between RANKL and IFN- γ on activated CD4 T cells¹⁶⁾. Dex induced human osteoclastogenesis without the addition of osteoblasts or soluble RANKL. The role of RANKL in this osteoclastogenesis process was confirmed by the inhibitory effect of OPG. Furthermore, Dex reduced the mean fluorescent intensity (MFI) of IFN- γ -positive CD4⁺ cells in a dose-dependent manner but did not affect that of RANKL-positive CD4⁺ cells. Interestingly, Dex increased the ratio of positive cells producing RANKL to those producing IFN- γ (RANKL:IFN- γ ratio) in a dose-dependent manner, suggesting that Dex stimulates osteoclast differentiation by elevating the RANKL:IFN- γ ratio in CD4⁺ T cells although the number of RANKL-producing cells is not changed. In addition, the production of IFN- γ from human activated CD4 T cells was reduced by Dex whereas the expression of mRANKL by RT-PCR was unchanged. Thus, it is speculated that the higher the RANKL:IFN- γ ratio of T cells only by reducing IFN- γ , the greater the number of osteoclasts induced by Dex *in vitro*. Further investigation is needed as whether Dex elevates RANKL:IFN- γ ratio in CD4⁺ T cells *in vivo*.

Conclusion

The direct effect of GC on bone metabolism appears to be more important than the indirect effects. On the basis of the above findings, GC appears to induce human osteoclastogenesis. Finally, we emphasize a potential novel mechanism whereby GC induces osteoclastogenesis via the up-regulation of RANKL and down-regulation of OPG or IFN- γ on osteoblasts and T cells. This finding suggests that the balance between RANKL and OPG or IFN- γ on osteoblasts and T cells may have an important role in GC-induced osteoclastogenesis. From a clinical perspective, the modulation of RANKL, OPG and IFN- γ expression by osteoblasts and T cells may represent a useful approach in the development of new treatment strategies to inhibit GC-induced osteoporosis.

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