Examination of Glucocorticoid Treatment on Bone Marrow Stroma: Implications for Bone Disease and Applied Bone Regeneration

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(ABSTRACT)

Long-term exposure to pharmacological doses of glucocorticoids has been associated with the development of osteopenia and avascular necrosis. Bone loss may be partially attributed to a steroid-induced decrease in the osteoblastic differentiation of multipotent progenitor cells found in the bone marrow. In order to determine if there is a change in the osteogenic potential of the bone marrow stroma following glucocorticoid treatment, Sprague-Dawley rats were administered methylprednisolone for up to six weeks, then sacrificed at 0, 2, 4, or 6 weeks during treatment or 4 weeks after cessation of treatment. Femurs were collected and analyzed for evidence of steroid-induced osteopenia and bone marrow adipogenesis. Although glucocorticoid treatment did inhibit bone growth, differences in ultimate shear stress and mineral content were not detected. The volume of marrow fat increased with increasing duration of treatment, but returned to near control levels after cessation of treatment. Marrow stromal cells were isolated from tibias, cultured in the presence of osteogenic supplements, and analyzed for their capacity to differentiate into osteoblast-like cells *in vitro*. Glucocorticoid treatment diminished the absolute number of isolated stromal cells, but did not inhibit the relative levels of bone-like mineral deposition or osteocalcin expression and secretion.

Although pharmacological glucocorticoid levels induce bone loss *in vivo*, physiologically equivalent concentrations have been shown to enhance the formation of bone-like tissue *in vitro*. However, glucocorticoids have also been reported to inhibit proliferation and type I collagen synthesis in marrow stromal cell cultures. In order to assess the effects of intermittent dexamethasone treatment on the progression of osteogenesis in rat marrow stromal cell culture, this synthetic glucocorticoid was removed from the culture medium after a variable period of initial supplementation. Cell layers were analyzed for total cell number, collagen synthesis, phenotypic marker expression, and matrix mineralization. Prolonged supplementation with

dexamethasone decreased proliferation, but did not significantly affect collagen synthesis. Furthermore, increased treatment duration was found to increase bone sialoprotein expression and mineral deposition. The duration of glucocorticoid treatment may be a key factor for controlling the extent of differentiation *in vitro*.