Dexamethasone Induced Adipogenic Differentiation and Expression of Adipokines and Cytokines in Mouse Bone Marrow Mesenchymal Stem Cells

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Introduction:
The association between corticosteroid administration and the development of osteonecrosis has been well established since 1957. However, the mechanism of action has not been elucidated. A number of investigators have studied the effect of dexamethasone on mesenchymal cell differentiation and the balance between adipogenesis and osteogenesis (1, 2). Adipocytes may also play a significant role in lipid metabolism (3) and have been shown to release a number of adipokines such as adiponectin, leptin, resistin, PAI-1, TNFα, and IL-6. Our study has focused on the changes of function of bone marrow progenitor cells in response to corticosteroid treatment in cultured mouse bone marrow mesenchymal stem cells.

Materials and Methods:
A pluripotent mouse bone marrow cell line, D1 (American Type Culture Collection, Manassas, VA) at passage 3 was seeded at a density of 3x10^5 per well in 12 well culture plates coated with 2ug/cm^2 mouse laminin. Cells were grown in complete growth media (DMEM with 10% FBS and 100U/ml penicillin and 100ug/ml streptomycin) under standard culture conditions (37°C; 5% CO2) and the medium was refreshed every 48 hours and experiments were started when cells reached 80% confluence. A final concentration of dexamethasone ranging from 10^{-4}-10^{-6}mol/L and 50ug/ml sodium ascorbate was mixed with complete growth media and was added into triplicate wells for 2, 4 and 6 days. The culture media was refreshed every 48 hours and the appearance of lipid droplets was monitored using a phase contrast microscope. The lipid produced by differentiating D1 cells was stained by Oil Red O and was measured by a triglyceride assay kit. Cells were harvested and cell culture supernatants were collected at each time point. The mRNA and protein expression of adiponectin, leptin, PAI-1, resistin, osteocalcin, HIF-1α, IL-6, TNF-α, VEGF and PPARγ was measured by real-time PCR and ELISA. The results were normalized to the control and GAPDH values and data were analyzed using the JMP (SAS) statistical package.

Results:
Dexamethasone treatment efficiently induces differentiation of bone marrow derived D1 cells into adipocytes as shown by Oil Red O staining and PPARγ expression. Triglyceride production has increased by 4 fold on day 6. Dexamethasone enhanced the release of adiponectin, resistin, osteocalcin, PAI-1, and HIF-1α in the culture medium starting from day 2. There were no detectable levels of IL-6, TNFα and leptin in the culture media collected at all time points. Expression of the gene PPARγ increased by 3 fold on the fourth day. Expression of VEGF mRNA decreased on the sixth day. Expression of mRNA of the inflammatory cytokines TNF-α, IL-6 and leptin has also increased on the sixth day of treatment.

Discussion:
Our study has focused on functional changes of bone marrow progenitor cells in response to corticosteroid treatment and whether the changes of gene expression and secretion of adipokines, within the bone marrow stroma contribute to the development of osteonecrosis. The effect of dexamethasone on the increase of osteocalcin expression may be due to the presence of a heterogeneous population of cells in the D1 culture and glucocorticoids are also known to promote osteogenic differentiation. Our results are in agreement with previous studies that glucocorticoids decrease angiogenesis by suppressing VEGF (2). Some of these adipokines such as PAI-1 and HIF-1α may also have an effect on the regulation of angiogenesis. Adiponectin and leptin are linked to the pathogenesis of hypertension, which involves, insulin resistance, endothelial dysfunction and vascular hypertrophy. Resistin has shown to increase transcriptional events leading to an increased expression of pro-inflammatory cytokines such as IL-1, IL-6 and TNFα. The cytokines are important positive or negative regulators of the cell cycle, cell differentiation and proliferation and also have a key role in cell-to-cell communication within the bone marrow stromal cell population.

Increased expression of IL-6 and TNFα has a pathogenic role in the abnormal bone resorption associated with osteoporosis, multiple myeloma, rheumatoid arthritis and other bone related disorders. Leptin has recently emerged as a mediator of the protective effects of fat on bone tissue. Leptin- induced MAP kinase activation may cause a reduction in the adipogenic differentiation by phosphorylating PPARγ and might play an important role in controlling adipogenesis. Although, several studies have measured adiponectin, leptin and resistin levels in plasma and adipose tissue, little is known about their changes of expression in bone marrow adipocytes. Altogether, our results confirm that continuous dexamethasone treatment will lead to an altered expression of a number of adipokines in the bone progenitor cells and raise the question of the interaction between adipokines - could a highly expressed adipokine modify the expression of others? Further studies are needed to answer this question and clearly understand the molecular mechanisms involved in steroid- induced osteonecrosis.

References:
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