Dexamethasone Treatment of Adipocytes Derived from a Mesenchymal Stem Cell Line Alters mRNA and Protein Expression of Key Adipokines in a Model of Glucocorticoid-Associated Osteonecrosis and Osteoporosis

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Introduction: Osteoporosis and osteonecrosis are strongly associated with glucocorticoid treatment, but the pathophysiology is unclear. The process is multi-factorial involving direct effects on bone multicellular units, along with altered coagulation, angiogenesis and endothelial responsiveness. High dose corticosteroid use (>2 g of prednisone, or its equivalent, within a period of 2 to 3 months) is a risk factor and up to 40% of these steroid users may develop osteonecrosis (ON) (Mont et al., 2006). Chronic corticosteroid use is also associated with osteoporosis.

Adipocytes are the most abundant stromal cells in adult human bone marrow but their role is disputed. They had previously been postulated to be simply space fillers and "support cells", or localized energy depots. However, bone marrow adipocytes hypertrophy in response to corticosteroids and may have significant regulatory effects on bone viability and healing by releasing signaling molecules called, "adipokines". This study examines the expression patterns of seven key adipokines in response to dexamethasone treatment in vitro in order to better understand the pathophysiology of glucocorticoid-associated osteoporosis and osteonecrosis.

Methods: The adipokines examined in this study were chosen because of their presumed role in the pathogenesis of steroid induced osteonecrosis and osteoporosis. They included hypoxia inducible factor 1 alpha (HIF-1 - an important transcription factor that regulates the body’s response to hypoxia), adiponectin and leptin (the two main circulating peptides from adipocytes), vascular endothelial growth factor A (VEGF-A - important in neovascularization and fracture healing), the antithrombotic plasminogen activator inhibitor 1 (PAI-1), Interleukin 6 (IL-6) and Tumor Necrosis Factor-Alpha (TNF-α) which is a pro-inflammatory, anti-adipogenic cytokine.

The mouse "D1" mesenchymal stem cell (ATCC) line were grown in DMEM (ATCC) + 10% FBS + Pen/Strep (Gibco) on Laminin coated plates (BD Biosciences) and differentiated in vitro with rosiglitazone (Cayman Pharm) into mature adipocytes. These mature adipocytes were then treated with varying concentrations of dexamethasone (Sigma): Control, 10^{-7} M 10^{-8} M, 10^{-9} M, 10^{-10} M; for 2, 4 or 6 days. After treatment, the mRNA from each sample was extracted with TRIzol Reagent (Invitrogen) and used for cDNA synthesis (ThermoScript RT-PCR System-Invitrogen) and the relative gene expression was measured using semi-quantitative real time PCR (iQ SYBR Green Supermix with the BioRad iCyycler) with gene specific primer pairs. PCR runs were performed in triplicate on triplicate RNA samples. The data was collected as relative fold change of mRNA expression in response to dexamethasone treatment of mature adipocytes results in:

1) HIF-1α mRNA and protein expression decreased significantly at 2, 4 and 6 days.
2) PAI-1 had no significant change in mRNA expression of but increased protein concentrations at 2, 4 and 6 days.
3) VEGF mRNA expression increased at 4 and 6 days with increased protein concentration at 4 days.
4) IL-6, TNF-α and leptin mRNA expression decreased at 4 and 6 days (with undetectable protein levels).

5) Adiponectin mRNA expression increased at 4 and 6 days (with undetectable protein levels).

Examples of mRNA and Protein Changes

Discussion
Dexamethasone treatment of adipocytes in vitro alters expression of key adipokine signaling molecules, which may play an important role in the pathophysiology of glucocorticoid-associated osteonecrosis or osteoporosis. Dexamethasone concentration appears to be less important than duration of treatment on gene expression.

Conclusions
Dexamethasone impairs hypoxia- inducible factor-1 expression in response to dexamethasone and may impair the local response to hypoxia and could potentiate any vascular compromise in osteonecrosis. This effect has been shown previously in cultured hepatocytes (Wagner2008), but never before in adipocytes. Increased PAI-1 protein levels are consistent with prior studies (Kerachian 2009) and may play an important role in intravascular coagulation in osteoporosis. The increase in VEGF mRNA expression is counterintuitive based on prior reports (Li 2005) which show that corticosteroids inhibit VEGF. However this study was done on undifferentiated cells whereas our study was done using mature adipocytes. TNF-α normally acts as an inhibitor of adipocyte proliferation and the significant decreases we found in TNF-α mRNA in response to dexamethasone could allow for the increased numbers of marrow adipocytes seen in osteonecrosis and osteoporosis. Likewise the increase in adiponectin and decreased leptin could result in decreased bone mass by increasing RANK-L expression and decreasing new bone formation respectively.

References:
1) Mont MA, Jones LC and Hungerford DS Nontraumatic Osteonecrosis of the Femoral Head: Ten Years Later: JBJS Am 88:1117-1132 2006

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