Corticosteroids Suppress TGF-beta/BMP Ligand Expression in Articular Chondrocytes

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Disclosures:

Introduction: Osteoarthritis (OA) is a common and debilitating disease that affects approximately 30% of the US population and is also a major clinical problem in companion animals. Many drugs are used to control the symptoms of OA, improve function and quality of life. Of these, intra-articular corticosteroid administration is a common and very effective anti-arthritis therapy. Corticosteroids exert their potent anti-inflammatory effects by blocking phospholipase A and reducing inflammatory mediator production; however, they also have several deleterious sequelae. Of particular relevance to the progression of OA, corticosteroid suppress the matrix-biosynthetic activities of articular chondrocytes. This activity, along with the increased joint use that symptomatic relief allows, has been linked to ‘steroid arthropathy’; a progression of arthritis driven by compromised chondrocyte homeostatic capacity. Several lines of experimental and clinical evidence emphasize the importance of TGF-b and BMP autocrine/paracrine activity in maintaining the homeostatic status of articular chondrocytes (1). We hypothesized that the suppressive effects of corticosteroids on articular chondrocyte matrix synthesis are mediated by down-regulation of TGF-b and/or BMP expression. The study was carried out to address the following objectives: 1) To assess the effects of corticosteroids on expression of chondro-protective TGF-b and BMP ligands in equine articular chondrocytes, and 2) To determine if exogenous TGF-b and/or BMP ligand administration can mitigate the suppressive effects of corticosteroids on articular chondrocyte synthesis of cartilage matrix.

Methods: Articular cartilage was collected from clinically normal joints of adult horses, euthanased for reasons other than musculoskeletal disease. Articular chondrocytes were isolated by overnight collagenase digestion and cultured as non-adherent aggregates in serum-free medium under non-adherent conditions. Triamcinolone acetate (TA) or methylprednisolone acetate (MPA) was added to the articular chondrocyte cultures at 10-100M, 10-7M, and 10-5M concentrations; comparable to in vivo exposure concentrations, for 72 hours. Effects of corticosteroid treatments on BMP and TGF-b ligand expression were assessed by qPCR. The effects of exogenous BMP and TGF-b ligand co-administration on collagen type II (Coll II) synthesis were assessed by ELISA. BMP and TGF-b ligand expression levels were determined by qPCR. To determine if exogenous TGF-b and BMP ligands have a direct effect on TGF-b and BMP expression, the effects of exogenous TGF-b and BMP ligands in equine articular chondrocytes, and 2) To determine if exogenous TGF-b and/or BMP ligand administration can mitigate the suppressive effects of corticosteroids on articular chondrocyte synthesis of cartilage matrix.

Results: Both BMP-2 and BMP-7 mRNA levels were significantly down-regulated by both TA and MPA administration, by 90% and 80% respectively. In contrast, BMPs-4 and 6 were not affected at any of the corticosteroid doses tested. TGF-b1 mRNA levels were significantly suppressed (by 60%) by both corticosteroids at all doses tested. There was no obvious dose-dependency, in that all three concentrations of TA and MPA down-regulated expression of these ligands equally. Somewhat surprisingly, TGF-b2 expression was increased by TA administration, though not significantly, while TGF-b3 expression was not affected. Exogenous BMP-2 administration (100 ng/ml) increased Coll II and aggrecan mRNA expression in the presence of MPA (Figure 1) but this effect on transcript levels was not reflected in any significant changes in secretion of Coll II or sGAGs by corticosteroid-treated chondrocytes. TGF-b1 administration (10 ng/ml) had no effect on Coll II mRNA levels and slightly down-regulated aggrecan expression. Coll II protein and aggrecan/sGAG secretion by corticosteroid-treated chondrocytes was not affected by exogenous TGF-b1.

Discussion: Both TA and MPA down-regulated BMP-2, BMP-7 and TGF-b1 mRNA expression in articular chondrocytes. These corticosteroid-mediated effects appear to be gene-specific, since BMPs-4 and 6, and TGF-bs 2 and 3 were not similarly affected. Although exogenous BMP-2 administration increased Coll II production under control conditions, this did not effectively prevent corticosteroid-mediated suppressive effects on cartilage matrix synthesis. These results suggest that other elements of the BMP and/or TGF-b signaling pathways articular chondrocyte are also impacted by corticosteroid administration, or that corticosteroids also suppress other metabolic pathways required for collagen and aggrecan processing and secretion.

Significance: The significance of this study lies in the recognition that several TGF-b/BMP ligands known to be critical for articular chondrocyte homeostatic activities are targeted by corticosteroids. This transcriptional suppression likely contributes to the untoward consequences of intra-articular corticosteroid delivery. Identifying alternative corticosteroids that maintain their anti-inflammatory activities without suppressing TGF-b/BMP ligand expression will reduce the negative consequences of these therapeutic agents. Accepting this, the inability of exogenous ligand administration to mitigate the suppressive effects of corticosteroids indicates that other critical bio-synthetic pathways are also impacted.

Acknowledgments:
References: 1. Oshin AO, Stewart MC (2007) The role of bone morphogenetic proteins in articular cartilage development,
Figure 1. Response of MPA-treated articular chondrocytes to co-administration of TGF-β1 (10 ng/ml) and BMP-2 (100 ng/ml). Collagen type II (Coll II) and Aggrecan mRNA levels were measured by qPCR. Significantly different expression levels, in comparison to the ‘MPA-only’ cultures (normalized to a value of ’1’), are indicated by asterisks (P<0.05; n=3).