CORTICOSTEROIDS SUPPRESS THE DIFFERENTIATED PHENOTYPE OF ARTICULAR CHONDROCYTES

Introduction:

Loss of articular cartilage is a characteristic feature of disease progression in osteoarthritis and is mediated in part through synovial membrane inflammation. The common clinical use of corticosteroids is based not only on the ability of these compounds to relieve pain and other symptoms of joint disease, but also on their ability to down-regulate the synthesis of matrix metalloproteinases and limit matrix protein degradation (1,2). However, enzyme-catalyzed matrix degradation is not the only important variable. New synovial membrane will not form until the capacity to synthesize new matrix is regained. Depressed production of matrix proteins in articular cartilage may also result from changes on the synthetic side of the equation. The purpose of this study was to examine how corticosteroids affect cartilage matrix protein synthesis by articular chondrocytes. The experiments were designed to test the hypothesis that therapeutic use of corticosteroids can alter chondrocyte function and change expression patterns of matrix proteins.

Methods:

Articular cartilage samples were collected from 10 equine ponies between the ages of 2.5 and 3.5 years of age with body weights between 200-300 kg. All experimental protocols were reviewed and approved by the institutional animal care and use committee. Right and left carpi of each pony were assigned to 1 of 4 experimental groups: control, steroid-treated, inflamed, and inflamed with steroid treatment. E. coli lipopolysaccharide (LPS) was used to induce synovitis by injecting 0.5 mg into the left radiocarpal and midcarpal joints every other day for a total of 4 injections. In 5 ponies, 0.1 mg/kg of methylprednisolone acetate (MPA) was administered into the radiocarpal and midcarpal joints of both forelimbs concurrently with the last dose of LPS. This dose was selected to approximate levels of MPA used clinically. The other 5 ponies did not receive MPA. The ponies were humanely euthanized by lethal pentobarbital injection 48 hours after the last intraarticular injection.

For in vitro experiments, chondrocytes were isolated from the articular cartilage of a 2-year-old horse by overnight incubation at 37°C in 0.075% collagenase type CLS1 from Clostridium histolyticum. Isolated chondrocytes were seeded in monolayer culture at a density of 5x10^5/cm² and maintained in Ham's F12 medium supplemented with 10% (V/V) fetal bovine serum. Methylprednisolone succinate (MPA) was added to the culture medium at concentrations ranging from 10^-2 to 1.0 mg/ml for up to 72 hours to evaluate the effect of corticosteroids on articular chondrocyte function and viability. Total RNA was isolated with modifications (3) to a commercial protocol using 32 P-labeled cRNA probes (4). Data were quantified from Northern blots and RNase protection assay gels by direct measurement of 32 P decay events using a Fujix Bio-Imaging Analyzer system. Statistical analyses of data collected from the in vivo experiments were based on mixed model analysis of variance due to confounding of treatment with individual ponies. The data were log₁₀ transformed to satisfy requirements of approximately equal variance and normal distribution. If a significant effect (p<0.05) of joint treatment was found, linear contrasts were used to determine the dose-response relationship. Analysis based on Pearson’s correlation coefficient was used to test for significant (p<0.05) differences in mRNA levels. For chondrocyte cultures, analysis of variance for repeated measures based on a Tukey multiple analysis was used to test for significant (p<0.05) differences in mRNA levels. 54 ± 3

Discussion:

Corticosteroids appear to directly suppress the differentiated phenotype of articular chondrocytes. Expression of type II procollagen and the (V+C)-containing fibronectin isoform (3) are markers of chondrocyte differentiation, both of which were significantly down-regulated by methylprednisolone. Cell culture data, combined with parallel changes induced by MPA in both normal and inflamed joints, suggest the corticosteroids work directly on the chondrocytes.

Potential clinical relevance of these findings is based on the widespread use of corticosteroids for rheumatic and many other conditions. Although clearly helpful in relieving symptoms of synovitis and suppressing excess metalloproteinase activity, these data support the premise that corticosteroids can damage cartilage independently of a concurrent disease process. Pathological changes noted in the articular cartilage of previously normal joints following corticosteroid administration include: loss of basophilia, decreased intensity of Safranin O staining, chondrocyte necrosis, and increased water content. These degenerative matrix changes appear to result in articular cartilage that is more susceptible to mechanical injury both in vitro (5) and in vivo (6). The observation that type II procollagen expression is significantly suppressed by even a single intra-articular injection of MPA, suggests a possible molecular mechanism for this structural vulnerability.

Considered in conjunction with their anti-inflammatory properties, corticosteroids clearly have the potential not only to alter the rate of proteinase catalyzed degradation, but also the rate and pattern of matrix protein synthesis by chondrocytes. Full assessment of how a given corticosteroid treatment protocol impacts articular cartilage should consider both of these parameters.

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References: