ADENOSINE PATHWAYS MODULATE CHONDROCYTE MEDIATED CARTILAGE DEGRADATION

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Introduction: The purine base adenosine (ADO) can function as an extracellular signaling molecule and has been reported to reduce inflammation in several in vivo models (1). Adenosine receptor stimulation increases intracellular accumulation of cAMP and suppresses nitric oxide (NO) synthesis by lipopolysaccharide (LPS)- and interleukin-1 (IL-1)-stimulated chondrocytes in monolayer culture (2,3). In addition, chondrocytes can be stimulated to accumulate extracellular ADO by exposure to LPS, while exposure to the adenosine kinase inhibitor, 5'-iodotubercidin (ITU), results in increased levels of extracellular endogenous ADO. The goal of this study was to investigate the potential beneficial effects of exogenous ADO supplementation and enhanced endogenous ADO levels in an in vitro model of articular cartilage degradation. In addition, we evaluated the role of endogenous adenosine in maintaining cartilage matrix homeostasis by depleting extracellular adenosine levels with adenosine deaminase (ADA).

Methods:
Cartilage Isolation and Treatment: Articular cartilage was obtained from the metacarpophalangeal joints of 11 fresh equine cadavers (1 to 7 years of age), and established in weighed explant cultures 48-hours prior to initiation of treatment protocols. Explants were cultured in basal medium consisting of Dulbecco's modified Eagle's Medium-Ham's F-12 nutrient mixture supplemented with 5% fetal calf serum (FCS) and appropriate antimicrobial agents.

In a set of experiments designed to investigate the potential protective effects of adenosine, LPS (50 µg/ml) or IL-1 (10 ng/ml) was added to treated explants to induce cartilage degradation. These explants were simultaneously exposed to ADO (100 µM), the adenosine kinase inhibitor ITU (1 µM) or both ADO and ITU. In a second set of experiments designed to investigate the role of endogenous adenosine in maintaining cartilage matrix homeostasis, explants were cultured in the presence of ADA at concentrations ranging from 0.5 to 2 U/ml. Controls included explants cultured in basal medium alone (control) as well as explants cultured with ITU and ADO without LPS or IL-1.

Assays: Following three days of exposure to the specified treatments, proteoglycan, NO, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and matrix metalloproteinase-3 (MMP-3) released into conditioned media were measured as indicators of cartilage metabolism and degradation. Glycosaminoglycan release was measured using a 1,9-dimethylmethylene blue assay. Nitric oxide levels were estimated by measuring nitrite concentration using a spectrophotometric assay based on the Griess reaction. Total MMP-3 concentration and PGE<sub>2</sub> levels were assayed in the conditioned media using enzyme-linked immunosorbent assays (R&D Systems).

Statistical Analysis: All values are expressed as mean ±SEM. Data were analyzed using a one-way ANOVA (P<0.05). Post-hoc means comparisons between control and treatment measurements were performed using a Dunnett's test (P<0.05).

Essential Results: As previously documented, IL-1 and LPS caused a significant release of proteoglycan, NO, PGE<sub>2</sub>, and MMP-3. The addition of ADO, ITU, or ADO with ITU each significantly inhibited LPS- or IL-1-induced proteoglycan release [Figure 1], PGE<sub>2</sub> release and NO production, but did not inhibit MMP-3 production. Addition of ADO or ITU in the absence of LPS or IL-1 did not have any detectable effect on cartilage metabolism in this model. Specifically, no increase in proteoglycan, NO or MMP-3 release was detected in cultures exposed to these treatments alone when compared to control cultures. In addition, previous cell viability studies have shown no adverse effects from exposure to ADO, ITU or ADA at the concentrations used here.

Exposure to ADA resulted in a concentration-dependent increase in proteoglycan release [Figure 2], NO synthesis, total MMP-3 concentration and PGE<sub>2</sub> release [Figure 3].

Discussion: Evidence is accruing that modulation of adenosine pathways has an anti-inflammatory action in arthritic conditions (1). The results presented here indicate that these pathways may also protect against cartilage damage induced by inflammatory mediators such as LPS and IL-1. The adenosine pathways may play significant roles in both physiological homeostasis and pathological disturbances in cartilage metabolism, and modulation of adenosine levels or the use of adenosine receptor agonists in the joint may have important therapeutic potential.