ARTICULAR CHONDROCYTES RELEASE ADENOSINE IN RESPONSE TO LIPOPOLYSACCHARIDE AND THE ADENOSINE KINASE INHIBITOR 5'-IODOTUBERCIDIN

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Introduction:
Adenosine (ADO) has received attention as a potential anti-inflammatory agent. The release of ADO from neutrophils has been postulated to influence the action of anti-rheumatic drugs but ADO release from resident articular cells has not been investigated. Chondrocytes express transcripts of receptors specific for ADO and respond to ADO receptor stimulation with an increase in intracellular cyclic-AMP accumulation and inhibition of lipopolysaccharide (LPS) and cytokine-induced nitric oxide. The purpose of the present study was to determine whether chondrocytes release ADO and whether release can be modulated by LPS, the adenosine deaminase inhibitor erythro-9-((2-hydroxy-3-onyl) adenine hydrochloride (EHNA), or the adenosine kinase inhibitor 5'-Iodotubercidin (IODO).

Methods:
Cell Isolation and Plating - Full-thickness equine articular cartilage was aseptically dissected from fresh equine cadavers. Chondrocytes were released by a three-step enzymatic digestion and freshly isolated chondrocytes were plated in 24-well plates at a density of 5 x 10^4 cells/cm².

High Performance Liquid Chromatography - ADO was detected by reverse-phase high performance liquid chromatography using a HP 1100 series. An aliquot of 300µl of tissue culture supernatant was mixed with 150µl of 4% trichloroacetic acid, incubated for 30 min at 4°C and centrifuged at 7500 RPM for 4 minutes. Samples were applied to a C18 column, 4.0 x 150mm with a 5µm particle size. Samples were eluted with a linear concentration of 0.1M KH₂PO₄ (pH 5.53) to 50% of 0.1M KH₂PO₄ 45% methanol (pH 5.58) over 21 minutes with a 1.0 ml/min flow rate. ADO was detected by retention time and concentration was calculated by comparison to standards.

DNA assay - DNA content per well was determined using Hoechst dye in combination with a 96-well fluorescent plate reader. Fluorescence was measured at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

Statistical Analysis - All values are expressed as mean ± S.E.M. Data were analyzed using a one-way analysis of variance (ANOVA) (p < 0.05). For time course experiments, comparisons between control measurements and treatment measurements (Dunnett’s test) were performed. When EHNA and IODO were tested together, each pair was compared using a student t-Test. Both tests were performed using JMP statistical software (SAS Institute).

Results:
Effects of LPS on ADO Release - Articular chondrocytes cultured in tissue culture medium alone did not release a measurable amount of ADO at any of the time points tested (data not shown). Chondrocytes released measurable levels of ADO, however, when exposed to 100µg/ml LPS (Fig 1).

Effects of an Adenosine Kinase Inhibitor on ADO Release - Chondrocytes released a measurable amount of ADO when exposed to the adenosine kinase inhibitor IODO (10µM) at times of 20 minutes or greater (Fig 2). Measurable ADO release from articular chondrocytes increased in a time-dependent manner in response to IODO.

Discussion:
ADO is released from cells under conditions of cellular stress and acts as a local modulator of tissue function. The enzyme adenosine kinase regulates intracellular metabolism of ADO while ecto-nucleotidases and adenosine deaminase rapidly degrade endogenously released ADO. Both ADO receptor agonists and adenosine kinase inhibitors attenuate inflammation in animal models, and ADO released from neutrophils have been shown to play a role in the actions of anti-rheumatic drugs. Our data shows that chondrocytes themselves can be stimulated to release ADO by LPS and IODO and that availability of extracellularly released ADO can be increased by the adenosine deaminase inhibitor EHNA. Autocrine release of ADO from chondrocytes may play a role in the cellular response to tissue damage in arthritic conditions. Pharmacological modulation of these pathways in the joint may have therapeutic potential.

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