**INTRACELLULAR SIGNALING BY MECHANICAL STRAIN INITIATES REPAIR AND REGENERATION IN CHONDROCYTES: IMPLICATIONS IN ORTHOPAEDIC REHABILITATION.**

Agarwal S; Gassner R J; Long P; Piesco N P. University of Pittsburgh, Pittsburgh, PA

**Introduction.** Osteoarthritis (OA) and rheumatoid arthritis (RA) are diseases of a complex etiopathology associated with progressive inflammation and cartilage destruction. Significant efforts made to assist OA and RA patients with non-invasive rehabilitative therapies have shown that physical therapies, such as continuous passive motion (CPM) and exercise, exert positive effects on diseased or inflamed synovial joints. However, neither the basis for the success or failure of CPM therapy is known, nor are the intracellular mechanisms of actions as yet revealed.

For the optimal application of orthopaedic rehabilitation, it is essential to first understand the molecular basis for its functional effectiveness. Inflammatory cytokines like IL-1beta play a major role in cartilage destruction, therefore, it was our hypothesis that mechanical strain exerts its beneficial effects via down-regulation of proinflammatory cytokine signal transduction pathways. In this report we have examined the intracellular actions of mechanical tensile strain (TENS) of various magnitudes on chondrocytes, to reveal the molecular basis for the reparative actions of CPM and exercise.

**Methods.** Chondrocytes were harvested from articular cartilage of rabbit knees and shoulders following IACUC approval from the University of Pittsburgh. Chondrocytes were grown in Ham's F-12 medium containing 10% fetal calf serum and were used within the first three passages. Cells were grown on flexible bottom Bioflex-II plates to 80% confluency and subjected to various magnitudes of equibiaxial tensile strain at 0.05 Hz in a Flexercell system (McKeepson, PA). Inflammation was mimicked in vitro by exposing cells to IL-1beta. To examine the effects of TENS on chondrocytes during inflammation cells were subjected to (a) untreated control, (b) TENS alone, (c) IL-1beta alone, or (d) IL-1beta and TENS (1, 2). The expression of proinflammatory molecules such as matrix metalloproteinase-1 (MMP-1), MMP-3, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) was assessed by reverse transcriptase/polymerase chain reaction (RT/PCR) and Western blot (WB) analysis. Generation of nitric oxide (NO) was assessed by Griess reaction, and prostaglandin E2 (PGE2) synthesis was measured by radioimmunoassay (Amersham Searl, IL). To assess the anabolic effects of TENS, proteoglycan synthesis was measured by radioimmunoassay (Amersham Searl, IL) and immunofluorescence. All experiments were carried out in triplicate and each experiment was repeated at least 3 times. Statistical analysis was carried out by ANOVA and the differences were considered statistically significant at $p<0.05$.

**Results.** Examination of the expression and synthesis of proinflammatory molecules by RT/PCR and WB revealed that control untreated chondrocytes do not express these molecules. However, exposure of these cells to IL-1beta resulted in a marked upregulation of MMP-1, MMP-3, COX-II, and iNOS mRNA expression, which was paralleled by production of their respective proteins, as well as PGE2 and NO production. More importantly, TENS between 3% and 8% elongation markedly down-regulated (by 38% to 92% as compared to cells treated with IL-1beta alone) IL-1? eta-dependent transcriptional regulation of proinflammatory genes. Furthermore, TENS at these magnitudes down-regulated IL-1beta induced IL-1, IL-6 and IL-8 mRNA expression and synthesis. These observations suggest that TENS acts upstream of mRNA induction via suppression of IL-1? signal transduction pathways.

We observed that mechanical signals are also reparative and augment matrix synthesis in chondrocytes in the presence of IL-1beta. Furthermore, signaling by TENS acts through a pathway, that is dependent on activation of proinflammatory pathways. TENS-dependent anti-inflammatory actions are mediated by inhibition of IL-1? -induced nuclear translocation of NF-/? B subunits, p50 and p65. The inhibition of NF-/? B nuclear translocation is due to TENS-dependent suppression of proteolytic degradation of I-kB? eta but not I-kB? lpha, the inhibitory units of NF-/? B. This causes suppression of NF-/? B nuclear translocation which is responsible for transcriptional activation of pro-inflammatory genes. However, the presence of an inflammatory signal was a prerequisite for the observed mechanno-transduction signals, as TENS alone did not induce nuclear translocation of NF-/? B. Functional analysis showed that the actions of mechanical strain do not involve IL-1 receptor down-regulation. We believe these findings are the first to demonstrate the molecular basis of mechanical strain as an important signal for cartilage repair and regeneration.

**Discussion.** We demonstrated that TENS of low magnitude is an effective antagonist of IL-1?B actions on chondrocytes. Intracellular actions of CTS are mediated through transcriptional regulation of multiple genes activated by IL-1?B. Furthermore, CTS actions involve disruption / regulation of critical step(s) in the signal transduction cascade of IL-1?B. By down regulating induction of catabolic proteins as well as up-regulating induction of extracellular matrix proteins, CTS not only acts as an anti-inflammatory signal but also as a reparative signal in IL-1?B-treated chondrocytes. Nevertheless, presence of IL-1?B is a prerequisite for these actions of CTS. As a potent antagonist of IL-1?B, actions of CTS on chondrocytes appear to be remarkably similar to those of current therapeutic agents that are being implemented to minimize cartilage degradation, such as anti-IL-1 immunoglobulins, IL-1 receptor antagonist, or metalloprotease inhibitors. This correlates with the well known effects of continuous passive motion in the augmentation of speedier and more physiologically sound recovery in orthopedic patients.


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