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**Angiotensin-converting enzyme inhibitors prevent osteoarthritis in a rat model**

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**Purpose:** The current study has been designed to investigate whether angiotensin-converting enzyme (ACE) inhibitors might prevent osteoarthritis in a rat model. In previous studies of our group, we have demonstrated that ACE inhibitors prevent loss of proteoglycan from cartilage samples in vitro. As osteoarthritis is typified by loss of proteoglycan, a drug that can delay proteoglycan loss might be of interest.

**Methods and Materials:** Induction of osteoarthritis in 350-gram Wistar rats was achieved by partial medial meniscectomy performed under general anesthesia. Two groups were evaluated. There were 10 rats in each group. In the control group, meniscectomy alone has been performed. In the experimental group, enalaprilate (5 mg/ml) was mixed with chitosan carrier gel and implanted into the joint prior to wound closure. Animals were sacrificed after 6 weeks and after 3 months. The joints were processed for routine histology. The parameters evaluated included a Mankin knee cartilage score, amount of proteoglycans assessed using image analysis of alcian blue stained slides and synovitis score.

**Results:** A significant difference in the degree of cartilage damage was observed in the experimental group as compared to the control group (4.5 ± 1.1 vs. 8.9 ± 2.2, t-test p<0.05). The synovitis score (1-5 score) was similar in both groups (3.2 ± 1 vs. 3.4 ± 0.9). The average density of proteoglycans was 128 ± 12 in the control group versus 163 ± 14 in the experimental group (out of 256 grey levels, t-test p<0.05).

**Conclusions:** ACE inhibitors appear to prevent proteoglycan loss in a surgically-induced model of osteoarthritis. The meniscectomy model is well established as a model for progressive medial compartment osteoarthritis. The intervention used relied on slow release of enalaprilate from chitosan-based gel. It appears that osteoarthritis progression is slowed by this intervention. It remains to be seen in further studies whether the chitosan itself possesses an ameliorative role. Another important point to be investigated prior to human implantation, is whether established osteoarthritis is affected by this combination therapy.

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**Role of transglutaminase 2 in apoptosis induced by hydrogen peroxide in human chondrocytes**

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**Purpose:** Chondrocyte apoptosis has been implicated in the pathogenesis of osteoarthritis. Transglutaminase 2 (TGase 2), the expression of which is higher in osteoarthritis patients, has been shown to be up-regulated during apoptosis in many experimental models. This study investigated the expression and role of TGase 2 in human chondrocytes undergoing apoptosis induced by hydrogen peroxide (H2O2).

**Methods and Materials:** Human chondrocytes were obtained from the knee articular cartilage of patients undergoing total joint arthroplasty. Chondrocyte apoptosis was induced by H2O2 and was measured with Annexin-V flow cytometry, DNA Fragmentation ELISA and DAPI staining. Quantitative PCR, Western Blot, an in situ activity assay and immunocytochemistry were used to examine TGase 2 expression. The role of TGase 2 was determined by determining the difference in the level of apoptosis before and after the cells were treated with monodansylcadaverine (MDC), a competitive substrate for TGase 2.

**Results:** H2O2 induced the apoptosis of human chondrocytes in a dose- and time-dependent manner. The level of TGase 2 expression was higher in the chondrocytes undergoing H2O2-induced apoptosis. MDC increased the level of apoptosis in the H2O2-treated chondrocytes, which highlights the protective role of TGase 2 in human chondrocyte apoptosis.

**Conclusions:** TGase 2 expression is higher in human chondrocytes undergoing apoptosis, and TGase 2 has a protective effect against apoptosis. These results may raise the possibility of TGase 2 as a modulator of cartilage damage in osteoarthritis by offering protection against chondrocyte apoptosis.

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**Synovial fluid composition in relation to intra-articular knee joint disorders**

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**Purpose:** Cartilage homeostasis is regulated by cytokines in the synovial fluid (SF). Following knee trauma, the composition of SF may be changed leading to altered chondrocyte metabolism. Orthokin® is one of several therapies aiming to block the detrimental effects of IL-1 through IL-1 Receptor Antagonist (IL-1RA). We analysed cytokines in SF of patients with various stages of knee joint disorders, and after treatment with Orthokin.

**Methods and Materials:** SF was aspirated from knee joints of patients directly after intra-articular trauma (AT, n=3), from patients with OA (n=10), and of patients who had been treated with Orthokin® (n=5). Measurement of IL-1, IL-4, IL-6, IL-10, IL-13, TNF-alfa and IFN-gamma in the SF was performed using Multiplex ELISA assay.

**Results:** IL-6 was present in SF of all 3 AT patients (avg. 1329 pg/ml), in 5 OA patients (avg. 15 pg/ml) and in 4 Orthokin-treated patients (avg. 22 pg/ml). IL-10 was present in 3 AT patients (avg. 6.3 pg/ml) and in 6 OA patients (avg. 2.5 pg/ml). IFN-gamma was present in the SF of 2 AT patients (avg. 31 pg/ml) and in 3 OA patients (avg. 14 pg/ml). IL-1, IL-4, IL-13 and TNF-alfa were not detectable in any of these patients.

**Conclusions:** Determination of cytokine levels in SF may form the basis for intra-articular therapies to prevent or diminish cartilage degeneration. IL-6 seems to be elevated in the SF of patients after an acute knee trauma, as compared to the OA and Orthokin group. Evaluation of IL-6 and a wider range of cytokines may elucidate mechanisms of intra-articular pathology.