Controls with and without chondroplasty were prepared for both groups. Media was changed every two days and group 2 media was supplemented with either IgF-1 or JNK-II. Eight 1mm slices from representative condyles were cut with a precision saw. Fours slice each were used for the cell viability and depth of penetration assay.

**Results:** Group 1: IgF-1 (50ng/ml) showed a significant improvement (p=0.03) in PG on Day 2. JNK-II (25uM) displayed improved PG without significance (p=0.06) on Day 2.

Group 2: IgF-1 (25ng/ml) significantly enhanced PG on Day 3 (p=0.03). IgF-1 (50ng/ml) significantly inhibited PG on Day 1, 3 and 7 while JNK-II (25uM) significantly inhibited PG on Day 1. The average depth of penetration for the representative cartilages slice was 99um while the depth of cell death averaged 151um.

**Conclusions:** The data suggest that a higher IgF-1 concentration is required to elicit the cytoprotective effect if administered prior to chondroplasty and a lower concentration must be used if IgF-1 is administered after chondroplasty. At higher concentrations (post-chondroplasty), IgF-1 and JNK-II inhibited proteoglycan synthesis. This suggests that a threshold concentration is needed to elicit the cytoprotective effect but higher concentrations inhibit PG. The mechanism of how these agents work in protecting cells needs further elucidation. This was an in vitro study which contains inherent weakness of not having synovial factors in the milieu. Bovine cartilage was used in this experiment instead of human osteoarthritic or cadaver cartilage due to availability and tissue quality. Human osteoarthritic chondrocytes have the potential to react differently to the cytoprotective agents and thus must be evaluated. Young bovine cartilage was used since chondrocytes proliferate much faster in young animals and may also have a greater response to cytoprotective agent than older tissue. The superficial zone of cartilage cells (within 100um of the articular surface) has the highest level of cell proliferation. Since the depth of penetration in our experiment averaged 99um, the most active chondrocytes have been removed by chondroplasty. These data support the hypothesis that pre or post treatment of articular cartilage with IgF-1 increases metabolic activity and provides cytoprotection when performing chondroplasty under these conditions.

**P189**

**MOUSE CARTILAGE UNDER COMPRESSION: INDUCTION OF NUCLEAR FACTOR-kB AND EXTRACELLULAR SIGNAL REGULATED KINASE1/2 ACTIVITY AND MODULATION BY AVOCADO/SOYBEAN UNSAPONIFIABLES**


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**Purpose:** We studied the main intracellular signalling pathways known to be involved in the prodegradative process of matrix cartilage (MAP kinases and NF-κB) in chondrocytes stimulated with the proinflammatory cytokine interleukin-1 beta (IL-1β) or in cartilage explants submitted to a mechanical stress (1MPa, 0.5 Hz). Moreover, we studied whether Avocado-Soybean Unsaponifiables (ASU), a common drug used in Europe for symptoms in osteoarthritis (OA), could modulate these intracellular signalling pathways.

**Methods:** Mouse costal chondrocytes in monolayer primary culture stimulated with IL-1β (10ng/ml) or mouse costal cartilage explants under mechanical stress (MS) were used in this study. The chondrocytes or explants were incubated in presence or absence of ASU (10μg/ml) NF-κB pathway was assessed by IκBα expression, by nuclear translocation of NF-κB using p65 antibody, by Electrophoretic Mobility Shift Assay (EMSA), using p50 and p65 antibodies. MAP kinase (MAPK) pathways were assessed by using phospho-p38, ERK1/2 and SAP/JNK protein expression.

**Results:** IκBα expression is decreased by 70% in compressed cartilage (after 2 hours of compression). IκBα expression is also decreased by 72% in presence of IL-1β (as soon as 2 minutes after stimulation), in parallel with the translocation of the cytosolic p65 subunit to the nucleus. Moreover, the binding of the heterodimer p50/p65 to NF-κB responsive element is significantly increased after IL-1β treatment. Interestingly, ASU partially prevent IL-1β-induced degradation of IκBα by 39% and MS-induced degradation of IκBα by 28%.

IL-1β-induced binding of p50/p65 is significantly inhibited in presence of ASU, in parallel with an inhibition of the translocation of p65 into the nucleus. Whereas the 3 MAPK p38, JNK and ERK1/2 are activated in presence of IL-1β, ASU inhibited specifically the ERK1/2 pathway by 34%. A same profile was observed in MS-activated chondrocytes.

**Conclusions:** We show here that, along with IL-1β, MS is also a strong trigger for NF-κB and ERK 1/2 activation suggesting that these 2 pathways are mechanosensitive in chondrocytes. Moreover, our study shows that ASU inhibit NF-κB and ERK1/2 pathways.

**P190**

**THERMOGRAVIMETRIC INVESTIGATION OF NORMAL AND DAMAGED HUMAN HYALINE CARTILAGE**

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**Purpose:** The purpose of this study was to elucidate the importance of water content in contributing to disease progression and to establish the kinetic character of water loss effect of heating. Previously, water content has not been measured thermoanalytically in normal and degenerative human hyaline cartilage. Therefore a new thermogravimetric protocol had to be established before the detailed investigation could be performed. Most of the known changes in the extra cellular matrix in OA comes from animal models since human samples for investigation are not widely available for experiment. The specific causes of osteoarthritis are unknown, but are believed to be a result of both mechanical and molecular events in the affected joint. Thermogravimetry (TGA) is one of the oldest thermal analytical procedures and has been used extensively in the study of polymeric systems.

**Methods:** During arthroplasty procedures performed at the Orthopaedic Department, University of Szeged, Hungary, degenerative human hyaline cartilage was obtained from 28 hip and normal cartilage from 7 knee. The samples were taken under sterile conditions, and excess bone was removed. Preoperatively the diagnosis of the patient was established on basis of the patient history, clinical signs and radiological findings. The state of the hyaline cartilage was determined intraoperatively. 35 samples were collected. Based on the patient diagnosis, seven samples were analyzed as normal hyaline cartilage, 12 were obtained from patients with femoral head necrosis, and 16 were collected from osteoarthritic cartilage. The thermogravimetical analysis was performed with the use of a MOM Derivatograph (MOM, Budapest, Hungary), and the TG, DTG and DTA curves were determined.

**Results:** It was found, that the total water content of intact (healthy) cartilage was 80.79% (SD: 7.09%), of necrotic femoral head was 87.80% (SD: 8.06%), of the osteoarthritic samples was 86.71% (SD: 7.84%). To remove the cartilage extra cellular water content 52.33% (SD: 6.68) kJ/M energy was needed in normal samples, in aseptic femoral head necrosis needed 70.25 kJ/M
energy and 72.72 kJ/M energy was used in case of osteoarthritic samples. Loss of water content in all three groups are presented with a sharp step on the TG curve, starting on average temperature of 37 °C and ending at 116 °C. Linear part of the TG curve begun at around 62 °C and ended at around 112 °C. loss. In case of the normal hyaline cartilage 1.266%/1°C fluid loss was detected. In necrotic samples 1.689%/1 °C decrease in mass was observed. In the osteoarthritic 1.422%/1 °C mass reduction was measured

**Conclusions:** Increase in the cartilage matrix water content in all cases of degenerative articular cartilage was observed. Based on the results it can be stated that water content is higher in impaired samples, meanwhile water interstitial bonding was stronger in these cases. Activation energy correlated considerably with water content in the samples. The newly established thermogravimetric protocol was sufficient for compositional thermoanalytical study of normal and degenerative human hyaline cartilage. Previously, this method has not been used for this purpose. Characterization of the altered metabolism in cartilage that promote disease progression should lead to future treatment options that can prevent structural damage. Therapeutic steps can be adequately tested and monitored with thermogravimetric measurements.

**P191**

**EVIDENCE FOR CLEAVAGE OF TYPE II COLLAGEN BY CATHEPSIN K IN HUMAN OSTEOARTHRITIC CARTILAGE**

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**Purpose:** Cathepsin K is expressed in normal and osteoarthritic (OA) hyaline cartilage and is capable of cleaving type II collagen as well as other matrix molecules. The aim of this study was to determine whether there is evidence for cathepsin K-mediated cleavage of type II collagen in human OA cartilage.

**Methods:** Femoral condylar cartilages removed at arthroplasty for knee OA were cultured in serum-free medium in the presence and absence of a synthetic cathepsin K inhibitor (supplied by Merck Frosst, Montreal, Quebec, Canada). The content of a new type II collagen cleavage neoepitope that can be generated by cathepsin K was measured by ELISA assay. Aggrecan degradation was measured by the release of glycosaminoglycan using a colorimetric assay. Inhibitor toxicity was assessed by measuring the incorporation of [3H]proline in cartilage cultured with and without the inhibitor. Type II collagen cleavage was also detected by ELISA and immunohistochemically in uncultured cartilages from both normal and OA knee joints.

**Results:** Cleavage of type II collagen was significantly enhanced in OA cartilage compared with healthy cartilage, as demonstrated by ELISA and immunolocalization. The inhibitor reduced collagen cleavage in cultures of 4 out of 8 patients, this being significant in 3 cases. There was no effect on proteoglycan release and the incorporation of tritiated proline was unaffected by the inhibitor.

**Conclusions:** These results show that cleavage of type II collagen at a site cleaved by cathepsin K is increased in OA articular cartilages. Based on the specificity and lack of detectable toxicity of the inhibitor, this cleavage is due in part to cathepsin K in almost half of the patients. Cathepsin K should therefore be considered as a potential therapeutic target in the control of cartilage degeneration in OA.

**P192**

**BOVINE, PORCINE AND ICHTHYIC CHONDROITIN SULFATE DECREASE IL-1beta EFFECTS ON NO PRODUCTION AND APOPTOSIS: CORRELATION WITH MOLECULAR MODELING DATA**

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**Purpose:** The current study examines whether porcine, bovine and ichthyic chondroitin sulfate (CS) would influence the production of nitric oxide (NO) and apoptosis in human osteoarthritic (OA) chondrocytes. Then, we confirm these results and explain them by a proposed novel activity concept of CS evaluated by molecular modeling.

**Methods:** Samples of human OA articular cartilage were obtained from patients undergoing knee arthroscopy. Firstly, OA human chondrocytes were incubated with porcine, bovine and ichthyic CS (100 μg/mL) and stimulated with human recombinant Interleukin-1 beta (hrIL-1 beta) (10ng/mL), in the same time, to induce NO synthesis. NO release was measured as nitrite concentration in 24 and 48 hours culture supernatants by using the Griess reaction. Secondly, OA human chondrocytes were incubated with porcine, bovine and ichthyic CS during 72 hours and stimulated by various concentrations SNP (Sodium Nitroprusside) during 18 hours. SNP was used as a NO compound donor. To access the degree of apoptosis, APOPercentage Apoplosis Assay was used. This finding was further quantitatively confirmed by fluorescent microscopy using two apoptosis markers: TUNEL assay and Annexin-V flus. In the third time, we modeled several CS oligosaccharides and we have tested their possibilities of interaction with IL-1 beta and its receptor.

**Results:** Bovine, porcine and ichthyic CS tested decreased significantly NO synthesis at 48 hours when human OA chondrocytes were cotreated with CS and hrIL-1beta. However, a preventive treatment with bovine, porcine and ichthyic CS during 72 hours and stimulation with hrIL-1beta did not reduce significantly NO synthesis. In OA chondrocytes treated with bovine, porcine and ichthyic CS, on average 18% of chondrocytes showed apoptotic features compared with 31% in chondrocytes treated with SNP. These data suggest that CS or CS oligosaccharides could interact with IL-1beta. The modeling study proposed two sites of interactions between IL-1 beta and some oligosaccharides. We have proved the specificity of this protein for distinct sulphatation sequences.

**Conclusions:** These results suggest that bovine, porcine and ichthyic CS prevent IL-1beta induced increase in NO production. This preliminary study suggests that bovine, porcine and ichthyic CS could downregulate apoptosis in the OA chondrocytes. A decrease of IL-1 beta effects could be the consequence of specific binding to oligosaccharides. This study provides a plausible mechanism for the chondroprotective properties of bovine, porcine and ichthyic CS.

**P193**

**CHONDROCYTE HYPTERTROPHY - A NOVEL EX VIVO MODEL FOR EARLY CHANGES IN CHONDROCYTES IN OA**

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**Purpose:** In early osteoarthritis (OA), hypertrophic chondrocytes are part of the pathology and are distributed throughout the