Lateral compartment was significantly different for each region (p < 0.001 for each; Figs. 2 & 3). In the medial compartment, the overall mean cellularity of the lateral compartment was 130 ± 0.06% that of the superficial zone only 36% that of the superficial zone. The cellularity of the medial periphery was 180% that of the center and midline (p < 0.001 for each; Figs. 2 & 3). Comparisons across regions found that the cellularity of the medial periphery was 180% that of the center and midline regions (p < 0.001).

Similarly, in the lateral compartment cellularity decreased greatly with increasing zonal depth (p < 0.001 for each comparison) with the cellularity of the deep zone only 36% that of the superficial zone. The cellularity of the lateral compartment was significantly different for each region (p < 0.02) with the mean cellularity highest in the periphery and least in the center region.

Conclusions: CE-CDPS is a new, structurally adjusted sulphated β-cyclodextrin derivative with preserved chondroprotective capacity and a promising safety profile.

235
REGION SPECIFIC CELLULARITY OF ARTICULAR CARTILAGE IN THE TIBIAL PLATEAU OF THE MATURE SPRAGUE-DAWLEY RAT
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Purpose: Despite the frequent use of the rat in models of osteoarthritis (OA), little has been reported regarding the normal cellularity of articular cartilage (AC) and how this varies regionally in the AC across the rat tibial plateau. In this descriptive study, we evaluated the regional and zonal-depth specific cellularity of AC in the rat tibial plateau in mature animals.

Methods: Tibial plateau from five male Sprague-Dawley rats approximately 12 months of age were collected. NIH guidelines for the care and use of animals were observed (n=10 specimens). Specimens were formalin fixed, decalcified with 10% EDTA, and paraffin embedded. Serial, 5 μm-thick, coronal sections of the posterior half of the tibial plateau were deparaffinized and stained with Hematoxylin and Eosin prior to examination under light microscopy. Region specific (periphery, central and midline) measures were determined by dividing each compartment (medial and lateral) into 3 equally spaced regions (Fig. 1). Depth specific measures (superficial: 0-25%, mid: 25-50% and deep: 50-100%) were determined by dividing the AC thickness into three zones from the surface to the tidemark. Chondrocytes with visible nuclei were counted in each sub-area for each compartment. The cellularity of the AC (chondrocyte number/AC area) was determined for each sub-area. Three slides were evaluated for each leg. Analysis of variance was used to compare mean cellularity across compartments (medial, lateral), regions (periphery, center, and midline) and zonal depths (superficial, mid, deep). Post-hoc pairwise comparisons were performed using Fisher’s LSD procedure.

Results: The overall mean cellularity of the lateral compartment was 130% that of the medial compartment (p<0.004). Cellularity varied significantly by depth and region within both the medial and lateral compartments (p<0.001 for each; Figs. 2 & 3). In the medial compartment, the overall cellularity progressively decreased with increasing depth from the surface (p<0.001 for each comparison). Comparisons across regions found that the cellularity of the medial periphery was 180% that of the center and midline regions (p<0.001).

Similarly, in the lateral compartment cellularity decreased greatly with increasing zonal depth (p<0.001 for each comparison) with the cellularity of the deep zone only 36% that of the superficial zone. The cellularity of the lateral compartment was significantly different for each region (p<0.02) with the mean cellularity highest in the periphery and least in the center region.

Conclusions: Compartment, regional and depth specific variations in AC cellularity were observed in the tibial plateau of mature Sprague Dawley rats in this comprehensive evaluation. The lateral compartment had a higher cellularity than the medial compartment and in both compartments cellularity was the highest in the superficial zone and the periphery region. Since all animals in this study were of the same age it is not known if some of these variations in cellularity may have developed with aging. These observed variations in cellularity across the tibial plateau may cause regional variations in response to experimental interventions. Care should be exercised to compare similar locations across animals in experimental studies.

236
CHANGES IN THE ENDOPLASMIC RETICULUM AND GOLGI COMPLEX FROM CHONDROCYTES ARE RELATED WITH THE OSTEOARTHRITIS PATHOGENESIS
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Purpose. The aim of this study was to identify changes in the endoplasmic reticulum (ER) and Golgi complex (G) from chondrocytes of the three zones of the whole cartilage since early stages of Osteoarthritis (OA) pathogenesis. Methods. The experimentally OA-induced model was accomplished by unilateral knee meniscectomy and post-surgery training; normal rats were used as a control. Animals were sacrificed by CO2 overdose and right femoral condyles were removed and processed for either electron microscopy (EM) or Immunohistochemistry (IHC). Structural changes in the ER and G from chondrocytes were identified at 1, 2, 3, 4 and 5 training days (td) by EM. In addition, changes in the protein expression levels of ER (calnexin) and G markers (58-k9 protein) were evaluated by IHC at 5, 10, 20 and 45 td. Results. During early stages of OA, chondrocytes undergo changes at morphological as well as at ultrastructural levels. These changes started in the superficial (SZ) and middle zones (MZ), showing a prominent ER development with expanded cisterns and enhanced G membranes. At the same time, chondrocytes acquired a rounded form and showed cells associations.
THE DYSFUNCTION OF THE MITOCHONDRIAL RESPIRATORY CHAIN REGULATES THE METALLOPROTEINASES EXPRESSION IN HUMAN NORMAL CHONDROCYTES IN CULTURE

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Purpose: Mitochondria is acquiring an important role in the progression of osteoarthritis (OA). Previously we have demonstrated that the alteration of the mitochondrial respiratory complexes III and V contributes to the inflammatory answer of the chondrocyte. Nevertheless, the possible implication of this organell in the process of destruction of the cartilage is not well defined. In this study, we have investigated the relationship between the dysfunction of the mitochondria and the possible modulation of extracellular matrix components in human normal chondrocytes in culture.

Methods: Human normal chondrocytes were isolated from cartilage obtained from autopsies without history of joint disease. Rotenone, NPA, antimycin A (AA), azide and oligomycin were employed to inhibit the mitochondrial complexes I, II, III, IV and V, respectively. MMP-1, -3 and -13 mRNA expression was studied by real time PCR. Intracellular protein expression was evaluated by western blot as well as by immunohistochemistry. Protein production was evaluated by ELISA. Proteoglycan presence was analyzed by alcian blue and safranin O stains.

Results: We treated cells with all the MRC inhibitors observing an up-regulation of MMP-1 and -3 mRNA expression at 24 hours of treatment with oligomycin 5 μg/ml (MMP-1: 68 ± 10 vs. basal=1; MMP-3: 60 ± 29.7 vs. basal=1). MMP-13 decreased after treatment with AA 60 μg/ml and oligomycin to 0.34±0.1 and 0.67±0.3 vs. basal=1, respectively. Also, we observed an increase in intracellular protein levels of MMP-1 and -3 after treatment with oligomycin 25 μg/ml. At 24 hours: 15.20±8.46 and 4.59±1.83 times vs. basal=1, respectively (n=4; *P<0.05). However, AA and oligomycin decreased the intracellular protein levels of MMP-13 (0.70±0.16 and 0.3±0.24, respectively vs. basal=1). In addition to this, levels of MMPs in the supernatants were evaluated. At 36 hours, MMP-1: 18.06±10.35 for oligomycin 25 μg/ml vs. basal=1, and MMP-3: 8.49±4.32 for oligomycin 5 μg/ml vs. basal=1 (n=5; *P<0.05). MMP-13 levels in the supernatants decreased after treatment of chondrocytes with AA 60 μg/ml (0.50±0.13 vs. basal=1) and oligomycin 25 μg/ml (0.41±0.14 vs. basal=1). (n=5; *P<0.05). The stimulation of tissue explants with the MRC inhibitors, showed an increase in the positive cells for MMP-1 and -3 after oligomycin treatment. By the same manner, stimulated punches with AA or oligomycin revealed a decrease in the MMP-13 expression. Alcian blue and safranin O stain, showed a loose of proteoglycans in tissues that were incubated with oligomycin.

Conclusions: These results show that the dysfunction of MRC modulates the MMPs expression in human normal chondrocytes.

MECHANICAL STRESS INCREASED P21(CIP1) EXPRESSION IN CHONDROCYTES

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Purpose: The cyclin-dependent kinase inhibitor p21(CIP1) was initially identified as a potent inhibitor of cell cycle progression. Subsequent studies further identified that p21(CIP1) has an important role in controlling cytostasis and cell death. p21(CIP1) transcription is activated by p53, and p21(CIP1) is part of a negative feedback mechanism that controls p53 activity during apoptosis. We have shown that mechanical stress induced chondrocytes apoptosis, and inhibition of p53 activation prevented chondrocyte from apoptosis induced by mechanical stress. Recently, Olive et al. reported that p21(CIP1) activity was essential for the regulation of cell proliferation and inflammation after arterial injury in local vascular cells. Further, p21(CIP1) regulated the expression of SDF-1 and MMP-13. These molecules are believed to be onset of osteoarthritis (OA) in articular cartilage. In this study, we analyzed the expression of p21(CIP1) in OA and normal chondrocytes. Furthermore, we evaluated the expression levels of p21(CIP1) in response to shear stress.

Methods: Normal cartilage samples were obtained from femoral head of patients (n=5) undergoing joint replacement surgery for the neck fracture of the femur. OA cartilage samples were collected from patients (n=8) during total knee joint replacement surgery. Endogenous p21(CIP1), p53, SDF-1 and MMP-13 mRNA were quantified by quantitative PCR and normalized to levels of 18S RNA. Values were average mRNA levels in OA chondrocytes/normal chondrocytes. Mechanical stress was introduced to NHAC-kn (cell line derived from human normal chondrocyte). Values were mRNA levels after loading 5%, 10% shear stress for 12h, 0.25Hz in comparison with control (non-stress).

Results: The expression levels of p21(CIP1), p53, SDF-1, MMP-13 were much higher in OA chondrocytes than in normal chondrocytes (Table 1). The