which could indicate GOA. The background of GOA was investigated by epidemiological analysis of peri-articular osteophytes of major six joints in a skeletal population.

070

AGE-RELATED BEHAVIOR OF COLLAGEN AND PROTEOGLYCAN IN SUPERFICIAL, MIDDLE AND DEEP ZONES OF PATELLAR CARTILAGE: FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS OF NORMAL RABBITS

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Purpose: To detect the collagen content and proteoglycan content in the superficial, middle and deep zones of articular cartilage is a trend but still technical challenge. Fourier transform infrared spectroscopy (FTIR) is powerful tool to investigate the collagen and proteoglycan in cartilage matrix. The FTIR quantifies the collagen content and proteoglycan content on histologic sections of cartilage. Because the FTIR maps the distribution of collagen and proteoglycan on the sections, the collagen and proteoglycan can be analyzed in the superficial, middle and deep zones of cartilage. Age-related behavior of the collagen and proteoglycan in each zone of patellar cartilage has not been reported yet. Therefore, the objective of this pilot study is to investigate the behavior of the collagen and proteoglycan in each zone using the FTIR.
Methods: Non-treated five rabbits of various ages (3-week, 8-week, 6-month, 1-year, 2.5-year) were examined. Specimens of patella were removed from each rabbit and all specimens were observed macroscopically and confirmed that the cartilage surface was smooth and glossy. Specimens were then prepared and sagittal sections were cut for histology and for the FTIR. The sections stained with safranin-O/fast green were observed using a light microscopy and confirmed that no fibrillation or no degenerative change had occurred. The sections for the FTIR were not stained. The integrated area of collagen Amide I (wave, 1590-1720 cm^{-1}) was defined as collagen content. The integrated area of proteoglycan sugar ring C-O absorbance (wave, 985-1140cm^{-1}) normalized by Amide I area was defined as proteoglycan content. The superficial, middle, deep and whole zones of cartilage were defined as the area from surface to 100 μm depth, 100 μm to 400 μm, 400 μm to 600 μm and surface to 600 μm, respectively. Mean collagen content and mean proteoglycan content of the each zone were calculated and compared.
Results: Collagen content in the superficial zone was the lowest in the 3-week animal and the highest in the 6-month animal (Fig. 1A). Collagen content in the middle, deep and whole zones was the lowest in the 3-week animal and the highest in the 2.5-year animal (Fig. 1A). Proteoglycan content in the superficial, middle and whole zones was the highest in the 6-month animal (Fig. 1B). Proteoglycan content in the deep zone was the highest in the 3-week animal (Fig. 1B). Proteoglycan content in the middle, deep and whole zones was the lowest in the 2.5-year animal (Fig. 1B).
Conclusions: The FTIR successfully detected the age-related behavior of collagen and proteoglycan in each zone of patellar cartilage. Because number of animals was limited, further study is needed.

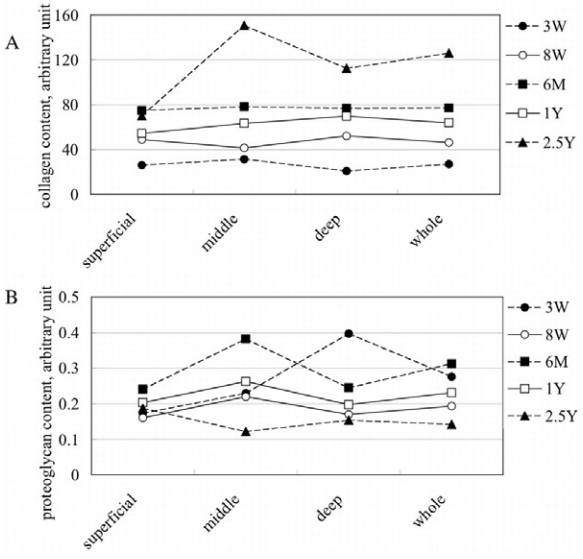

Figure 1. Age-related behavior of collagen content (A) and proteoglycan content (B) in each zone of patellar cartilage.

Animal models

071

ORAL TREATMENT WITH THE PLANT EXTRACT OF BRACHYSTEMMA CALYCINUM D. DON REDUCES THE DEVELOPMENT OF CARTILAGE LESIONS IN EXPERIMENTAL DOG OSTEOARTHRITIS: INHIBITION OF PROTEASE ACTIVATED RECEPTOR-2

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Purpose: There is an obvious need for safe and effective new agents that can stop the progression of osteoarthritis (OA). Botanical medicinal products or neutraceuticals used for the treatment of OA in general have been demonstrated to have better tolerability than classical drugs. J&L is a plant extract prepared from Brachystemma calycinum D. don, a plant classically used in Chinese medicine for the treatment of musculosqueletal diseases.
The aim of this study was to evaluate the potential protective effect of J&L on the in vivo development of OA lesions in the experimental dog anterior cruciate ligament (ACL) transection model and to document its mechanism of action.
Methods: OA was induced by sectioning the ACL of the right knee in crossbred dogs. There were two treatment groups (n=6-7 dogs/group): placebo and J&L at a therapeutic dosage (200 mg/kg/day), given orally for the entire duration of the study (8 weeks). Macroscopic and histopathological evaluations of cartilage lesions on the femoral condyles and tibial plateaus were performed. Moreover, immunohistochemical analyses of cartilage assessing the levels of iNOS, MMP-13 and protease activated receptor (PAR)-2 were done.
Results: Treatment with J&L reduced the severity of cartilage OA lesions. More specifically it reduced the depth of cartilage lesions on the tibial plateaus with a significant effect on the femoral condyles ($p \leq 0.04$). J&L treatment significantly decreased ($p \leq 0.02$) the histopathological score on both femoral condyles and tibial plateaus; the main effect was observed on structural changes and cellularity. J&L also significantly reduced the level of key inflammatory and catabolic factors, iNOS ($p \leq 0.009$) and MMP-13 ($p \leq 0.003$), as well as the level of PAR-2 ($p \leq 0.03$).
Conclusions: This study demonstrates that the J&L can have a protective effect on the development of experimental OA articular (or joint) structural changes. This effect was associated

with the inhibition of major catabolic and inflammatory mediators. This study is the first to demonstrate that therapeutic treatment inhibiting PAR-2 can be associated with a disease-modifying OA effect.

072

AKT1 IN CHONDROCYTES CONTROLS CARTILAGE CALCIFICATION DURING SKELETAL GROWTH AND OSTEOPHYTE FORMATION IN OSTEOARTHRITIS

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Purpose: Endochondral ossification plays crucial roles in skeletal growth and osteoarthritis (OA) progression. Since the phosphoinositide-dependent serine-threonine protein kinase Akt is known to be a pivotal signaling molecule for several factors regulating cartilage metabolism, this study examined the possible involvement of Akt in the endochondral ossification process under physiological and pathological conditions.
Methods: Expressions of the Akt isoforms, chondrocyte differentiation and calcification markers, and inorganic pyrophosphate (PPi)-related factors were assessed by real-time RT-PCR or Western blotting in primary costal chondrocytes from neonatal mice or mouse chondrogenic ATDC5 cells. To know the in vivo role of Akt1, we compared the skeletal phenotypes between homozygous Akt1-deficient (Akt1-/-) mice and the wild-type littermates by radiological and histological analyses including HE, Safranin-O and von Kossa stainings, BrdU labeling, and immunostainings of type X collagen (COL10) and vascular endothelial growth factor (VEGF). An experimental OA model was created surgically by inducing instability in the knee joints, and OA severity was quantified by the OARSI histopathology grading system. For the functional analyses, we established stable lines of ATDC5 cells with retroviral overexpression of constitutively active Akt1 (ca-Akt1) or small interfering RNA of Akt1 (si-Akt1). Cell proliferation was assessed by CCK-8 assay. The chondrocyte differentiation was determined by Alcian blue staining and COL10 mRNA level under the stimulation of insulin, as well as luciferase assay using ATDC5 cells transfected with a reporter construct containing a COL10 promoter fragment. The cartilage calcification was assessed by Alizarin red and von Kossa stainings, and expressions of alkaline phosphatase, VEGF, and osteopontin under the stimulation of insulin and phosphate.
Results: Among the Akt isoforms (Akt1, 2 & 3) Akt1 was most highly expressed in primary chondrocytes, and both phosphorylated and unphosphorylated Akt proteins were considerably decreased in the Akt1-/- chondrocytes, indicating a major role of Akt1. The Akt1-/- mice exhibited dwarfism with shorter limbs and trunks than the wild-type littermates. In the Akt1−/- growth plate, BrdU-positive proliferative and COL10-positive hypertrophic zones were normal; however, cartilage calcification at the bottom by the von Kossa staining was significantly suppressed. Under the OA induction in knee joints of the two genotypes, articular cartilage degradation and chondrocyte hypertrophy were comparable, while osteophyte formation due to cartilage calcification was prevented in the Akt1-/− joints. In the ex vivo culture of Akt1-/- costal chondrocytes, although proliferation and differentiation were normal, calcification parameters were significantly suppressed compared to the wild-type culture. The ATDC5 cell culture confirmed that the calcification was significantly enhanced by overexpression of ca-Akt1 and suppressed by that of si-Akt1, while none of proliferation, differentiation, or the COL10 promoter activity was affected by the gain- or loss-of-function of Akt1. Among the principal regulators of PPi, a crucial inhibitor of cartilage calcification, expressions of PPi stimulators ANK and NPP1 were suppressed by ca-Akt1 and enhanced by the Akt1 deficiency or si-Akt1.
Conclusions: Akt1 in chondrocytes controls cartilage calcification by inhibiting PPi during endochondral ossification in the skeletal growth and in the osteophyte formation of OA. Elucidation of the signals related to Akt1 will lead to further understanding of the molecular background of OA.

073

EARLY DEGENERATIVE ARTICULAR CARTILAGE ALTERATIONS ARE RELATED TO SPECIFIC JOINT INJURIES IN AN OVINE MODEL OF OSTEOARTHRITIS

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Purpose: To characterize differences in histology, gross morphology, and osteophytosis in stifle joints from sheep that underwent either combined anterior cruciate/medial collateral ligament transection (ACL/MCLx), lateral menisectomy (Mx), or Sham surgery. The present study was based on the hypothesis that the location of focal articular cartilage degeneration will differ in the two injury models, which are both known to lead to osteoarthritis.
Methods: All surgical procedures were reviewed and approved by our Institutional Animal Care Committee. A total of 23 skeletally mature female Suffolk-cross sheep were allocated into four groups: ACL/MCLx (n=7), Mx (n=5), Sham (n=5), and NOC (n=6). Surgeries were performed on the hind right stifle and animals were sacrificed 20 weeks post-injury. At dissection, both hind stifles were examined and scored for gross degeneration and osteophytosis at 12 standardized regions using established scoring systems. Cartilage samples were then harvested from these regions, were stained with safranin O and fast green, and graded using a modified Mankin score. To moderate inter-animal variability, degeneration scores of the left stifle were subtracted from those of the right stifle, creating a 'normalized' degeneration score. Results for gross morphology, osteophytosis, and histology were analyzed separately.
Results: The contralateral (left) stifles were unremarkable with no significant differences between any of the groups. Normalized scores from each type of assessment were low: within 36% or less of the maximum possible degeneration score, with average scores of 17% or less. When scores across locations were summed to yield a composite grade of overall joint degeneration, only the Mx group had significantly more gross articular cartilage degeneration than the Sham and NOC control groups, but had more osteophytosis than any other group ($p<0.01$). Significant differences in histology were detected between groups, but post-hoc tests showed only the Mx exhibited a trend of worsened degeneration compared to Sham ($p=0.06$). Mankin scoring categories of structure, cellularity, cell cloning, and proteoglycan staining exhibited little differences between most groups, except the Mx group, which exhibited higher scores. Site-specific differences between groups existed in gross morphology and osteophytosis ($p<0.05$). Mx sheep had more degeneration and osteophyte formation within the lateral tibiofemoral compartment than other groups ($p<0.01$), whereas the ACL/MCLx group had much more variable responses, but generally tended to have more degeneration in the medial compartment. Although not statistically significant, site-specific histology demonstrated similar trends. There were no significant differences between Sham and NOC groups in any of the degeneration assessments.
Conclusions: In young, skeletally mature sheep, Mx tended to create more overall joint degeneration than ACL/MCL transection; this difference reached significance in osteophytosis scoring and showed a strong trend in histological changes. Moreover, the locations of gross focal articular cartilage degeneration and osteophytosis were different between the two injury models. Although these osteoarthritic lesions can only be considered to be