

cartilage behaviour was the Viscoelastic Lineal Model described by Hayes et al. (1972). Like this, the Poisson rate (ν), Young module (E) and Shear module (G) were obtained. Statistical for Windows: ANOVA with $p < 0,05$ was used to statistical analysis.

Results: The results can classify themselves in two groups: direct results (highest and equilibrium load); and indirect results (highest and equilibrium ν , E and G) (Table 1).

Table 1. Estatistical data found

Group	Pmax2	Peq2	Vmax	Ve _q	E _{max}	E _{eq}	G _{max}	Ge _q
16 weeks								
X	59,597*	1,073*	0,197	0,197	54,572*	3,446	22,796*	1,439
SD	21,749	0,473	0,000	0,000	28,586	3,796	1,941	1,586
19 weeks								
X	88,680*	2,547*	0,197	0,197	85,761*	3,837	35,843*	1,603
SD	16,772	1,260	0,000	0,000	20,189	2,843	8,433	1,187

X: median; SD: standard deviation; * $p > 0,05$.

Conclusions: This method allows obtain the necessary direct and indirect values to characterize the mechanical behaviour of articular cartilage in rabbit's femoral condyles, showing normal viscoelastic behaviour of the samples. The 19 weeks samples show higher values of instantaneous load, Young and Shear module than 16 weeks samples, because of the articular cartilage rigidity increase with the animal age. Similar results occur in the equilibrium load. The significant differences mean that this method is enough sensible to evaluate these samples.

P75

GENE EXPRESSION PROFILES OF NORMAL AND RUPTURED CANINE CRANIAL CRUCIATE LIGAMENT

D.N. Clements¹, J.F. Innes¹, S.D. Carter¹, W.E. Ollier², P.J. Day²

¹Faculty of Veterinary Science, University of Liverpool, Liverpool, United Kingdom, ²Centre for Integrated Genomic Medical Research, The University of Manchester, Manchester, United Kingdom

Purpose: Anterior cruciate ligament (ACL) rupture is associated with the development of knee osteoarthritis (OA). In the dog, a similar association between cranial cruciate ligament (CCL) rupture and stifle OA is also recognised. The aim of the study was to investigate if there were differences in gene expression between normal CCL and ruptured CCL. Gene expression in normal CCL was also compared between breeds of dog predisposed to CCL rupture and breeds of dog at low risk of CCL rupture.

Methods: Grossly normal CCL was harvested from the healthy stifles of 10 dogs euthanatized for welfare reasons, due to naturally occurring, non-orthopaedic disease (5 from breeds predisposed to CCL rupture and 5 from breeds protected from rupture). CCL remnants were harvested from the stifles of 5 dogs with naturally-occurring CCL rupture undergoing surgical treatment of the condition. mRNA was extracted, amplified, labelled and hybridized to a 44,000 gene canine whole genome oligonucleotide microarray chips. Array scan data were normalised and compared between the different groups. Differential expression of selected genes was confirmed by quantitative PCR (qPCR) using a larger number of CCL samples (21 ruptured, 13 normal [predisposed to CCL rupture], 7 normal [low-risk of CCL rupture]).

Results: When comparing the ruptured CCL to normal CCL, 99 transcripts were significantly up-regulated (including CTSK, COL3, CASP8), and 16 transcripts were significantly down-regulated. qPCR confirmed the increased expression of CASP8, COL3 in the ruptured CCL, and increased expression of additional genes including COL1, MMP2 and IGF1. No significant differences were identified between the gene expression profiles of the normal CCL of breeds predisposed to CCL rupture

when compared to the normal CCL of breeds at low risk of CCL rupture, either by microarray or qPCR.

Conclusions: Altered transcriptional activity in was identified in the ruptured CCL when compared to the normal CCL. A general pattern of up-regulation of expression of selected proteases and matrix-associated genes characterises the transcriptome of the ruptured CCL. Differences in the risk of CCL rupture between breeds of dog predisposed to CCL rupture when compared to breeds of dog at low risk of CCL rupture could not be related to changes in gene expression in the normal CCL.

P76

SUBCHONDRAL BONE CHANGE IS SECONDARY TO CARTILAGE DEGENERATION IN THE RAT MENISCECTOMY MODEL OF OSTEOARTHRITIS

H. Yasui, J. Hata, K. Yamana, H. Takagi, T. Jinbo, T. Shimomoto, D. Miura, Y. Harada, Y. Oue, Y. Azuma
TEIJIN Pharma Limited, Tokyo, Japan

Purpose: According to the clinical observation, there are two opposing hypotheses: bone sclerosis is secondary to cartilage loss and the result of cartilage breakdown; bone sclerosis precedes cartilage degeneration and loss. The purpose of this study is to investigate the relationship between articular cartilage degeneration and subchondral bone change in the rat meniscectomy model of osteoarthritis (OA).

Methods: The medial collateral ligament of right knee of rat was transected and the medial meniscus was resected (MNX). MNX rats were sacrificed at 1 - 7 weeks after the surgery (n=6 animals/group), and Sham-operated (SHM) rats at 4, 7 weeks. Three-dimensional (3D) structural change of tibial subchondral bone was evaluated using micro-focused X-ray computed tomography (micro-CT), followed by histological assessment.

Results: In the histological assessment, articular cartilage degeneration was observed from 1 week in MNX rats, and cartilage degeneration was progressed until 4 week in a time-dependent manner. In the histological scoring system, the score was increased between 1 and 4 week, and sustained up to 7 week in the MNX rats, but it was unchanged up to 7 week in the SHM rats. In the micro-CT analysis, subchondral bone was unchanged until 3 week, but subchondral bone plate and subchondral cancellous bone were thickened from 4 week at the medial tibial weight bearing region of MNX rats. As for the change of subchondral bone, bone volume fraction (BV/TV) and bone width were increased and bone surface/volume ratio (BS/BV) was decreased in a time-dependent manner. In SHM rats, subchondral bone was unchanged until 7 week in the micro-CT analysis.

Conclusions: In this study, subchondral bone change was developed later than articular cartilage degeneration in the meniscectomized rats. At first, articular cartilage degeneration was induced by meniscectomy, and mechanical stress was increased at bearing region of subchondral bone. Next, the change of subchondral was proceeded in the meniscectomized tibia. These data suggest that subchondral bone change was induced as the secondary to cartilage degeneration and loss in this condition.

P77

EVALUATION OF PARTIALLY-SELECTIVE MMP-13 INHIBITORS IN THE RAT MENISCAL TEAR MODEL OF OA

S.S. Glasson¹, J.I. Levin², A.M. Bendele³, M.A. Rivera-Bermudez¹, T.J. Blanchet¹, Q. Wang¹, E.A. Morris¹
¹Wyeth, Cambridge, MA, ²Wyeth, Pearl River, NY, ³BolderBioPath, Inc., Boulder, CO

Purpose: Published studies have demonstrated efficacy of a

broad-spectrum (MMP-2, -3, -8, -9 and -13) matrix metalloproteinase (MMP) inhibitor in the rat meniscal tear model of Osteoarthritis (OA). In order to assess if MMP-13 was a critical enzyme responsible for cartilage degradation in this model, a non-selective MMP inhibitor (MMPI) was compared to a more-selective inhibitor MMP-13 (MMP-13SI).

Methods: All animal studies were performed at Bolder BioPath with IACUC approval. Lewis rats were gavaged twice daily with vehicle, 30 mg/kg MMPI or 30 mg/kg MMP-13SI, starting the day prior to surgery, with 20 rats per group. Surgery consisted of sectioning of the medial collateral ligament and medial meniscus. At 3 weeks post-surgery, plasma was taken for PK analysis, rats euthanized and the knees harvested for histologic evaluation.

Results: The MMPI gave comparable efficacy to other broad-spectrum MMP inhibitors, and was consistent across 3 separate studies. PK analysis showed excellent exposure for both compounds. Despite excellent PK, the MMP-13SI showed no efficacy in this model.

Conclusions: Several studies using broad-spectrum MMP inhibitors (including MMP-13) have shown efficacy in-vivo, but none have been reported with selective MMP-13 inhibitors. This is the first published study to show that a more MMP-13 selective inhibitor, with good PK, was not efficacious in the rat meniscal tear model of OA.

P78

ANTINOCICEPTIVE ACTIVITY OF A POLYSACCHARIDE IN THE ANTERIOR CRUCIATE LIGAMENT TRANSECTION MODEL IS INDEPENDENT ON THE GEL STATE

F. A. Rocha Sr.¹, R.R. Castro¹, P.R. Cunha², J.A. Feitosa²

¹Faculty of Medicine, ²Chemistry Department, Federal University of Ceará, Fortaleza, Brazil

Purpose: To evaluate the effect of a polysaccharide derived from Guar gum (GG) given intra-articularly (i. art.) in joint pain in an osteoarthritis (OA) model in rats.

Methods: Wistar rats were subjected to ACLT (OA group). Joint pain was measured using the test for articular incapacitation, until 7d. The OA group was compared to false-operated (sham). GG (100µg), gel or solution, or Hylan G-F 20 (100µg) were given i.art. at day 4 after ACLT and joint pain was recorded daily, until sacrifice, at day 7. Controls received saline i.art.

Results: Animals of the OA group presented significant joint pain as compared to sham ($P < 0.001$). GG, either as a gel or solution, significantly inhibited joint pain. This effect was similar to the inhibition achieved with Hylan G-F20.

Conclusions: This is the first demonstration that a galactomanan derived from GG is antinociceptive in experimental OA. The antinociception is independent of the colloidal state. We propose that the analgesic benefit of viscosupplementation may be due to an intrinsic carbohydrate-mediated mechanism, rather than to the rheologic properties of the material.

Biomarkers

P79

MEASUREMENT OF SYNOVIAL FLUID VOLUME USING UREA

T. Stabler, J. Kong, G. Varju, G. McDaniel, V.B. Kraus
Duke University Medical Center, Durham, NC

Purpose: The purpose of this study was to examine the utility of using urea concentrations for determining synovial fluid (SF) joint volume.

Methods: Knee joint SF was collected either directly or by saline lavage from both knees of 140 human subjects. SF was aspirated directly from 165 knees and by lavage from 110 knees. No SF was obtainable from 5 joints. Serum was obtained immediately prior to SF aspiration. All participants met ACR and radiographic (KL grade ≥ 1) criteria for symptomatic OA of at least one knee. Subjects with knee replacement or bilateral knee OA of KL grade 4 were excluded. Participants were asked to rate individual knee pain, aching or stiffness as none, mild, moderate or severe. SF and serum urea levels were determined using a specific enzymatic method run on an automated CMA600 analyzer. Cell counts were performed on direct SF aspirates when volume permitted. The formula for calculating SF joint volume was as follows: $V_j = C_D(V_i)/(C - C_D)$ with V_j =volume SF in entire joint, C_D =concentration urea in diluted (lavage) SF, V_i =volume saline injected into joint, and C =concentration urea in undiluted (neat) SF derived below where $C = 0.897(C_s)$, C_s =concentration urea in serum.

Results: Subject ages ranged from 35-85 years with a mean of 64 and a median of 66. There was an excellent correlation ($r^2 = 0.8588$) between SF and serum urea in the direct aspirates (Fig. 1) with a ratio of 0.897 (SF/serum). As expected neither urea levels nor the SF/serum ratio showed any correlation with KL grade, or cell count. While urea levels increased with age there was no change in the ratio. Intraarticular SF volumes calculated for the lavaged knees ranged from 0.555ml to 71.71ml with a median volume of 3.048ml. There was no correlation of SF volume to KL grade but there was a positive correlation ($p = 0.001$) between SF volume and self reported individual knee pain (Fig. 2).

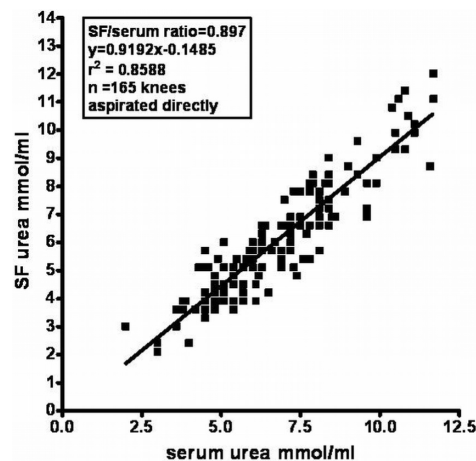


Fig. 1. Correlation of SF and serum urea in direct aspirates.

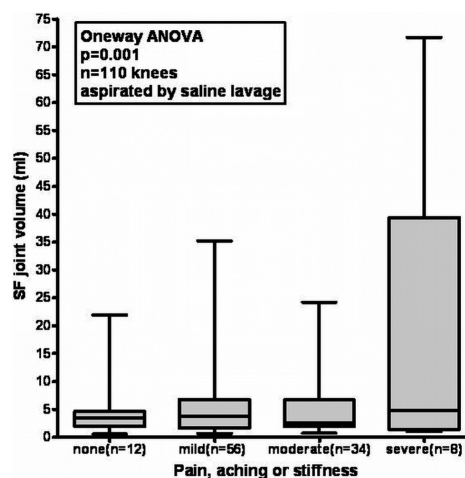


Fig. 2. Knee joint SF volume by pain.