

**Purpose:** Several predictive animal models have been developed for human osteoarthritis (OA) and used to study the preclinical *in vivo* efficacy of disease-modifying OA drugs (DMOADs). In these studies, the preclinical efficacy has been determined by using various microscopic scoring systems. Recently, the OARSI histopathology initiative presented recommendations for histological OA assessment in different species in order to standardize preclinical efficacy studies. In this study, we performed a systemic characterization of knee joints in four rat OA models by using OARSI rat scoring system in association with the assessment of OA-related joint pain.

**Methods:** The study was conducted using male Lewis rats. Unilateral OA was induced in knee joints at 3 months of age by applying the following rat OA models: 1) intra-articular injection of monoiodoacetate (MIA) at the dose of 1 mg/kg, 2) medial meniscal tear (MMT) combined with medial collateral ligament transection (MCLT), 3) anterior cruciate ligament transection (ACLT) combined with partial medial meniscectomy (pMMx), 4) ACLT. Body weight, static weight bearing determined as hind paw weight distribution, and static mechanical allodynia determined as paw withdrawal threshold were followed in each model during the study. Knee joints were harvested and digital radiographs were obtained from them at two different time points in each model as follows: in the MIA model at 2 and 4 weeks, in the MMT + MCLT model at 3 and 6 weeks, in the ACLT + pMMx model at 4 and 8 weeks, and in the ACLT model at 5 and 10 weeks. Microscopic assessment of degenerative changes was performed in knee joints as recommended by the OARSI histopathology initiative.

**Results:** Static weight bearing was decreased in operated knee joints during the first week of the study, demonstrating an operation-related joint discomfort. Paw withdrawal threshold was decreased in operated and MIA-injected knee joints during the first week and at the end of the study, indicating operation and OA-related joint pain. Microscopic assessment of OA demonstrated progressive degenerative changes in knee joints in all animal models. Mild to moderate changes were observed in the MIA and ACLT models. In the MIA model, mild degenerative changes included the loss of chondrocytes, proteoglycans (PG) and collagen matrix in superficial layer of articular cartilage in medial tibial plateau at 2 weeks, and the chondrocyte and PG loss exacerbated down to intermediate layer at 4 weeks. In the ACLT model, chondrocytes, PG and collagen matrix were lost mainly in superficial layer at 5 weeks, their loss exacerbated down to tidemark at 10 weeks, and these changes were associated with the presence of moderate to large osteophytes and minimal synovial inflammation. The MMT + MCLT and ACLT + pMMx models exhibited moderate to severe degenerative changes. In the articular cartilage of medial tibial plateau, chondrocytes, PG and collagen matrix were lost from superficial layer down to tidemark in the MMT + MCLT model at 3 and 6 weeks, and in the ACLT + pMMx model at 4 and 8 weeks. These changes were associated with the presence of large osteophytes and mild synovial inflammation in both models.

**Conclusions:** This study characterized progressive degenerative changes as recommended by the OARSI histopathology initiative in knee joints of four rat OA models used frequently in the preclinical *in vivo* efficacy studies of DMOADs. Mild to moderate degenerative changes were observed in the MIA and ACLT models and moderate to severe changes in the MMT + MCLT and ACLT + pMMx models. In all OA models, the development of degenerative changes was associated with decreased paw withdrawal threshold, indicating OA-related joint pain.

### 113 OLIGOMYCIN, AN INHIBITOR OF COMPLEX V OF MITOCHONDRIAL RESPIRATORY CHAIN, INDUCES AN INFLAMMATORY RESPONSE IN RAT KNEE JOINT

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**Purpose:** A decline of mitochondrial function has been described in OA chondrocytes and RA synoviocytes. Recent *ex vivo* findings support a connection between mitochondrial dysfunction and activation of inflammatory and destructive pathways in these cells. The aim of this study was to investigate *in vivo* articular model if the intraarticular injection of oligomycin, an inhibitor of mitochondrial function, induces a destructive and inflammatory response in rat knee joints.

**Methods:** 24 female wistar rats (180–220g) was divided into three study groups: Healthy (no intraarticular injection); Lipopolysaccharide (LPS)-treated, positive control (left joint injected with: LPS 10ug and right

joint with vehicle); and Oligomycin (OLI)-treated (left joint injected with Oligomycin 20ug and right joint with vehicle). Three intraarticular injections were carried out at days 0, 2 and 5. Rats were sacrificed at day 6, hind paws were collected and joint tissues were obtained. Measurement of joint diameters on stimulus- and control-injected paws was performed at days 0 and 6. Histopathologic lesions were evaluated by hematoxylin-eosin (H&E) and masson trichromic stain sections in synovial tissue and by safranin O staining in cartilage. By RT-PCR, CINC-1, IL-1 $\beta$ , CCL-2 and TNF- $\alpha$  gene expression were analyzed in extracted cartilage. And by immunohistochemical staining, IL-8, equivalent of CINC-1, expression was localized in the joint tissue.

**Results:** OLI-treated hind paws significantly increased the joint diameter (0.9 $\pm$ 0.1 mm, n=8, p<0.05, vs vehicle-injected joints), similarly to LPS-treated (2 $\pm$ 0.3 mm, n=8, p<0.05, vs vehicle-injected joints). In relation, histological evaluation of synovial tissue by H&E staining revealed that joints treated with mitochondrial inhibitor present greater synovial lining hyperplasia, proliferation of subsynovial tissue and infiltration of a marked number of inflammatory cells while the right control synovial only contained a moderate synovial proliferation and inflammation (3.3 $\pm$ 0.1 vs. 2.1 $\pm$ 0.2, respectively, n=8, p<0.001, vs vehicle-injected joints). Besides, immunohistochemical studies on IL-8 showed a greater expression in synovial tissue from OLI-injected joints versus those from vehicle-injected joints, coinciding with a strong neutrophils infiltration. In relation to cartilage, when the loss of matrix in the cartilage by safranin O staining was evaluated no differences were observed, neither when IL-8 immunoperoxidase staining was performed in cartilage. By contrast, when CINC-1 mRNA was analyzed in this tissue, it was detected a significant increment in OLI-injected joint (n=8, p<0.05 vs. vehicle-injected joint), similarly to LPS-treated.

**Conclusions:** The data seems to support that a loss of mitochondrial function in the joint could participate in rheumatoid pathology through generating an inflammatory response in the articular tissue, contributing to the perpetuation of joint injury.

### 114 EXPRESSION OF PPAR A, B, G, AND H-AND L-PGDS DURING OSTEOARTHRITIS IN THE SPONTANEOUS HARTLEY GUINEA PIG AND THE EXPERIMENTAL DOG MODELS

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**Purpose:** To investigate the expression of peroxisome proliferator-activated receptor (PPAR) a, b, g, and hematopoietic and lipocaline-type prostaglandin D synthase (H- and L-PGDS) over the course of osteoarthritis (OA) in the spontaneous Hartley guinea pig and the anterior cruciate ligament transection dog models.

**Methods:** Guinea pigs were sacrificed at 2 (control group), 4, 8, and 12 (n = 5 per group) months of age. Non-operated (control) and operated dogs were sacrificed at 4, 8 and 12 weeks post-surgery. Cartilage was evaluated histologically using the Osteoarthritis Research Society International (OARSI) guidelines. The expression of PPARa, b, g, and H- and L-PGDS were evaluated by real-time PCR and immunohistochemistry. The non-parametric Spearman test was used for correlation analysis.

**Results:** PPARa, b and g were detected in medial tibial plateau from control animals in both the spontaneous and surgical models. The levels of PPARa and b did not change over the course of OA, whereas PPARg levels decreased during the progression of the disease. We also showed that the expression of H-PGDS remained unchanged, whereas that of L-PGDS increased over the course of OA. PPARg levels correlated negatively, whereas L-PGDS levels correlated positively, with the histological score of OA.

**Conclusion:** The level of PPARg decreased, whereas that of L-PGDS increased during the progression of OA. These data suggest that reduced expression of PPARg may contribute to the pathogenesis of OA, whereas enhanced expression of L-PGDS may be part of a reparative process.

### 115 INITIATION AND PROGRESSION OF OCHRONOTIC OSTEOARTHROPATHY IN ALKAPTONURIC MICE

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