THE ROLE OF TRANSFORMING GROWTH FACTOR ALPHA IN A MOUSE MODEL OF OSTEOARTHRITIS

S.E. Usmani, C.T. Appleton, I.D. Welch, F. Beier
The Univ. of Western Ontario, London, ON, Canada

Purpose: Osteoarthritis (OA) affects approximately ten percent of all Canadians and is the leading cause of physical disability and impaired quality of life in North America. Despite the prevalence and severity of this degenerative joint disease, its underlying pathological mechanisms are not well understood. Recently, our lab established a surgical rodent model of OA in order to study gene expression changes in degenerating articular cartilage. We found that transforming growth factor alpha (TGF alpha) gene expression was upregulated in our model, and thus identified TGF alpha as a novel growth factor involved in OA. Further in vitro studies showed that TGF alpha treatment suppressed chondrocyte expression of the anabolic factors aggrecan and type II collagen and increased expression of the catabolic factor matrix metalloproteinase 13 (MMP13). The purpose of this study is to examine the requirement for TGF alpha in the progression of osteoarthritis in vivo. We hypothesize that Tgfa null mice will experience delayed OA progression compared to control littermates in a surgical disease model.

Methods: Ten week old Tgfa null mice and their heterozygous littermates underwent meniscotibial transaction (MTX) of the left knee joint in order to create a mild form of degenerative disease. Mice from both groups also received sham surgery (capsuleotomy). Seven weeks post-surgery, animals were sacrificed, and their knee joints were isolated and prepared for histology. Tissues were stained with safranin-O/fast green and joint histopathology was scored using the Osteoarthritis Research Society International (OARSI) criteria. Tissues were also immunostained for MMP13 expression.

Results: Preliminary results show that MTX appears to produce a very mild form of degenerative joint disease while sham surgery shows no sign of disease at seven weeks-post surgery. Importantly, Tgfa null mice have lower OARSI scores and express less MMP13 than their heterozygous littermates at this time point.

Conclusions: TGF alpha appears to play an important role in the progression of osteoarthritis in vivo. Future studies will examine the expression of additional anabolic and catabolic factors in Tgfa null joints as well as the potential role of TGF alpha in the cartilage growth plate.

IMPLEMENTATION OF A SCID ENGRAFTMENT MODEL WITH SYNOVIAL FIBROBLASTS AND CARTILAGE TO characterize OA patients

L.M. Gierman1, A. Koudijis1, M. Kloppenburg2, V. Stojanovic-Susulic3, G.J. Van Osch4, T.W. Huizinga2, A.-M. Zuurmond1

1TNO, Leiden, Netherlands; 2LUMC, Leiden, Netherlands; 3Centocor, Radnor, PA; 4Erasmus MC, Rotterdam, Netherlands

Purpose: Patient heterogeneity is probably one of the reasons for failure of osteoarthritis (OA) clinical trials. We aim at identifying novel biomarkers and disease mechanism by focussing on adipose and synovial tissue from OA patients for improved patient stratification

Methods: For this purpose, we implemented an engrafment model in the SCID mouse for in vivo characterization of synovial and adipose tissue from OA patients. The model was set up using synovial fibroblasts from rheumatoid arthritis (RA) patients that are known for their invasive behaviour. A sterile gelfoam sponge was seeded with synovial fibroblasts and co-implanted together with bovine cartilage. Evaluation of cartilage destruction and synovio-cyte invasion was performed 60 days after implantation by H&E and safranine-O staining and compared with cartilage implanted with synovial fibroblasts from healthy donors.

Results: Cartilage destruction and invasion of RA synovial fibroblast in the cartilage was observed.

Conclusions: This model will be used to study the effects of adipose and synovial tissue from OA patients on cartilage integrity and invasive behaviour.
Results: Following ACL section, limb impairment rapidly developed in all dogs, with PVF and GCA values dropping by week 4. After this acute disability phase, the dogs underwent a slow remission phase that was still incomplete by week 26. Prediction of PVF change was best estimated ($R^2=0.96$) from GCA and BML-SPGR ($p<0.0001$), particularly during the phase of acute disability, whereas cartilage defect was more influential ($R^2=0.97$) during the remission phase ($p=0.0051$) (week 8 to week 26). The other joint structural damages had significant impact on limb impairment and recovery. Both BML-SPGR and cartilage defect adversely affected the recovery in PVF, with mutually independent effects. Similar to ln_PVF, GCA showed an acute drop by week 4, followed afterwards by a remission phase ($p<0.0001$), which attained baseline values by week 26 ($p=0.46$). The time-course of GCA recovery was negatively affected by cartilage defect, and was positively affected by joint effusion ($p<0.0001$ for both variables).

Conclusions: In recent human OA studies, pain and limb impairement were related mostly to BML and joint effusion. Our data from dogs with experimental OA confirms the role of BML and joint effusion on limb function. On one hand, BML and cartilage defect hinder the recovery of PVF. On the other hand, joint effusion positively influences GCA, supporting the existence of alleviating mechanisms that oppose to abnormal biomechanics. This study also clarifies the role of cartilage and other joint structural components in OA: cartilage volume and osteophytosis act as confounding factors with negligible role in limb impairment. Such structure/function modeling opens promising avenues for assessing outcome of disease-modifying OA drugs at the preclinical development stage.

089

SELECTIVE AGGREGANASE INHIBITION IS DISEASE MODIFYING AND PAIN ALLEVIATING IN A RAT MENISCAL TEAR MODEL OF OSTEOARTHRITIS

S.S. Glasson1, A. Bendele2, P-E. Sum1, S. Tam1, J. Tejada1, M. Rivera-Bermudez1, J. Skotnicki3, E. Morris1, K. Georgiadis1

1Wyeth, Cambridge, MA; 2Bolder BioPath, Boulder, CO; 3Wyeth, Pearl River, NY

Purpose: The purpose of the study was to evaluate the efficacy of an ADAMTS-4 and ADAMTS-5 selective inhibitor in a robust animal model of osteoarthritis (OA).

Methods: All animal studies were performed after IACUC approval. Male Lewis rats (20 per group) underwent medial meniscal tear surgery in the right knee joint.1 Rats were treated twice daily by oral gavage with vehicle, selective ADAMTS-4/-5 inhibitor (ASI) (10, 30, or 100 mg/kg) or a broad-spectrum MMP inhibitor (30 mg/kg). All treatments were initiated 1 day before surgery and continued for 13 weeks. At the end of the dosing period, rats were euthanized and analyzed for pharmacokinetic analysis. Weight-bearing on the operated right leg and contra-lateral left leg was evaluated in the vehicle-treated and 30 mg/kg ASI-treated groups using an incapacitance meter, and expressed as grams difference in weight bearing between right and left leg. Following euthanasia, right knees were collected for histopathology evaluation including subjective and objective measures of cartilage degeneration and measures of joint capsule thickening.

Results: Statistically significant decreases in cartilage degeneration scores and width of severe lesions were observed in animals treated with both 30 and 100 mg/kg BID ASI. No adverse events were observed in animals treated with any dose of ASI. Broad-spectrum MMP inhibitor treatment resulted in decreased histologic evidence of cartilage degradation, but also caused a 40% thickening of the medial joint capsule, 6% lower body weight and a reluctance to mobilize. The vehicle-treated animals demonstrated a 27±2 grams decrease in weight bearing on the operated compared to the control limb, while the animals treated with 30 mg/kg SI had only a 16±2 grams decrease in weight bearing. This 41% increase in relative weight bearing on the unstable knee was statistically significant.

Conclusions: This study demonstrated that the significant reduction in cartilage degradation following surgical induction of joint instability observed in gene-deleted ADAMTS-5 and ADAMTS-4/5 mice can also be produced by small molecule inhibition of enzyme activity. There were no adverse physiologic effects following systemic inhibition of ADAMTS-4/5 activity for 13 weeks. The normalization of weight-bearing, after treatment with an efficacious dose of ASI, indicated that decreasing cartilage degradation as a result of inhibition of Aggrecanase-mediated aggrecan degradation, positively affected stance and pain. These results support this strategy of selective ADAMTS-4/5 inhibition as a safe and effective method of disease modification and pain modification of osteoarthritis, and should be evaluated in the clinic.

090

GAIT ANALYSIS AND BEHAVIOURAL PAIN RESPONSE OF TWO RODENT MODELS OF OSTEOARTHRITIS

C.E. Ferland, S. Laverty, F. Beauudy, P. Vachon

Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada

Purpose: To evaluate gait pattern recorded on the CatWalk and pain behaviour in two different rat models of osteoarthritis (OA).

Methods: Twenty-two male Sprague-Dawley rats weighing 200±25g were studied. Two weeks prior to the induction of OA, animals were trained on the CatWalk runway (Noldus) to traverse the corridor uninteruptedly. Mechanical allodynia was assessed by measurement of withdrawal thresholds in response to application of von Frey filaments. During the second week of the training period, data were collected to obtain baseline values. One group of rats (n=8) underwent surgical anterior cruciate ligament transection with partial medial meniscectomy (ACL+PMMx) to mimic a joint instability model and another (n=8) received an intra-articular injection of monooiodoacetate (MIA) (3mg/30μl) as an inflammatory pain model. After recuperation (2 days MIA, 5 days ACL+PMMx), the tests were performed for four consecutive weeks. After the behavioural measurement period, rats were sacrificed. Both knee joints were collected for histological assessment as well as spinal cord lumbar enlargements for neuropeptide analysis by HPLC/ESI/MS/MS. Repeated measure analysis of variances (linear model) followed by a sequential Bonferonni correction were performed for gait analysis parameters and von Frey results.

Results: No significant differences were observed in the gait speed between the three groups at each time point and in comparison with the baseline values. Changes in dynamic gait parameters were observed starting on the first day of testing, post OA-induction, in both models. A tendency towards stabilization in the surgical model was observed with parameters returning near to the baseline values (ex. swing phase duration, the swing speed and the ratio between the stance phase and the complete step cycle duration). These observations were seen in both hind limbs, with no statistical differences between the ipsilateral and the contralateral limbs. Conversely, in the MIA model significant changes remained in the injured limb compared to the contralateral limb in the swing phase duration ($p<0.02$) and the swing speed (p0.2). With von Frey filaments, mechanical sensitization was observed in the ipsilateral limb of the MIA model only ($p<0.0001$). Neuropeptide analysis demonstrated significant increase in CGRP concentrations in both models with 5803±1520 pmol/g and 4651±586 pmol/g in the ACL+PMMx and MIA models.