THE EFFECTS OF DELAYED ADMINISTRATION OF RHO-KINASE INHIBITOR FASUDIL ON SURGICALLY INDUCED OSTEOARTHRITIS IN RATS

M. Pest, V. Pitelka, F. Beier. Univ. of Western Ontario, London, ON, CANADA

Introduction: Osteoarthritis (OA) is a highly prevalent joint disease in North America. Current treatment methods focus on relief of symptoms including pain, with little to no effect on the underlying joint damage. Our studies have demonstrated that the signaling molecule transforming growth factor alpha (TGFα) is upregulated in animal models of OA and a subset of human cases. Downstream targets for TGFα include rho-associated protein kinase (ROCK). Inhibition of ROCK in cartilage explant cultures has been shown to decrease catabolic degradation of collagen II and aggrecan. Corroboration of these findings in an animal model would help to solidify ROCK as a potential drug target for drug studies in human tissue.

Purpose: To evaluate the protective effects of delayed administration of ROCK inhibitor fasudil (HA-1077) following surgical induction of rat OA in vivo.

Methods: OA was induced surgically in the right knee joint of male Sprague-Dawley rats by method of anterior cruciate ligament transection with partial medial meniscectomy; sham surgery serves as a control. Treatment began at 4 weeks post-surgery using osmotic pumps administering vehicle, 3 or 15 mg/kg/day of fasudil to groups of 5 rats each with an additional sham-operated control group. Groups were terminated at time points 3 and 6 weeks after initiation of treatment, with additional vehicle and sham groups sacrificed at 4 weeks post-surgery as comparisons. Development of OA was evaluated in safranin-O/fast green stained coronal knee sections using a modified OARSI scoring system.

Results: Rats treated with fasudil at a concentration of 3 mg/kg/day for 3 weeks exhibited statistically significant lower histologically assessed cartilage damage compared to vehicle and 15 mg/kg/day treatment groups; this group was also not statistically different from vehicle at 4 weeks post-surgery (0 weeks treatment). This effect was lost at 6 weeks of treatment. Treatment with 15 mg/kg/day fasudil showed no significant difference from vehicle at any time point, or difference from 3 mg/kg/day fasudil at 6 weeks treatment.

Conclusions: Treatment with a low dose of fasudil through subcutaneous osmotic pumps slowed the progression of cartilage damage at 3 weeks treatment time in rats with established OA, however this effect was lost at 6 weeks treatment. A higher dose does not seem to protect against cartilage degeneration at either 3 or 6 weeks treatment, which may be due to toxicity or other dose related effects.

128 IMPROVED ASSESSMENT BY QUANTITATIVE DIGITAL HISTOMORPHOMETRY OF HISTOPATHOLOGICAL CHANGES OF ARTICULAR CARTILAGE IN A SURGICAL MODEL OF POST-TRAUMATIC OSTEOARTHRITIS OF THE KNEE JOINT IN RATS

K.A. Rudolphi. Sanofi, Frankfurt-Main, Germany

Purpose: The goal of the study is to evaluate the development of the histopathological changes over time in a model of surgically-induced osteoarthritic knee joints in rats and to compare the most frequently used subjective semi-quantitative scoring of joint damage with computerized quantitative digital histomorphometry.

Methods: Male skeletally mature Lewis rats (12 weeks of age, n = 10-12 animals/group) were subjected to unilateral transection of the Anterior Cruciate Ligament plus 25% removal of the Medial Meniscus (ACLTpMx model) or sham operated. Three, 7, 28, 56 and 84 days following ACLTpMx the animals were sacrificed and in Hematoxylin-Eosin- and Safranin-O-stained (SO) coronal paraffin sections the degree of joint damage was evaluated by two observers in a blinded fashion using a modified histopathological Mankin score. For quantitative histomorphometry digital images of the joint were analyzed using the digital image analysis software Integrator VIS System Version Nr. 3.0.15.0 (http://www.visipharm.com Denmark). The degree of cartilage destruction and subchondral bone sclerosis was quantified by measuring the following parameters: 1. cartilage surface irregularity, 2. cartilage area, 3. chondrocyte number, 4. area of proteoglycan-containing (SO-stained) cartilage and 5. area of sclerotic subchondral bone.

Results: The histopathological changes (cartilage fibrillation and erosion, chondrocyte loss, proteoglycan depletion and subchondral bone sclerosis) developed rapidly with increasing severity over time. Already 28 days after ACLTpMx moderate to severe signs of OA were observed. Sham-operated animals did not develop significant OA pathology at any time point. The histomorphometric parameters showed a significant correlation with the corresponding Mankin-subscores.

Conclusions: The ACLTpMx model of OA in rats shows similar features as human knee OA regarding anatomical location and the specific histopathological morphology. Quantitative digital histomorphometry of cartilage destruction and subchondral bone sclerosis offers a more objective and less time consuming assessment of OA histopathology in this experimental model than classical histopathological scoring, thus facilitating the preclinical pharmacological testing of potential disease-modifying drugs.

129 GAIT ANALYSIS AFTER HYALURONIC ACID INJECTION INTO OSTEOARTHRITIC KNEE JOINTS OF MOUSE

Y. Muramatsu 1, T. Sasho 1, M. Saito 1, S. Yamaguchi 1, N. Ikegawa 1, R. Akagi 1, S. Mukoyama 1, A. Watanabe 2, Y. Wada 2, K. Takahashi 1,2. Dept. of Orthopaedic Surgery,Graduate Sch. of Med.,Chiba Univ., Chiba; 1 Dept. of Orthopaedic Surgery, Teikyo Univ. Chiba Med. Ctr., Ichihara, Japan

Background: Several experimental animal models of osteoarthritis(OA) have been developed to help our understanding of OA. They are especially useful in histological assessments of interventions against OA but behavioral analysis, which is another important aspect of OA feature, has sparsely been performed on them. Gait disturbance results from joint pain associated with OA and gait analysis would be important to evaluate the progression of OA as well as histological evaluation. In the present study, gait analysis was conducted with CatWalk systemTM developed for the use of small animals. It is an automated gait analysis system and has been validated as a method to quantify abnormal gait pattern in rat models of arthritic pain. But there has been no comprehensive analysis of its use in mouse model along OA development.

Purpose: The goal of the study is to evaluate the development of the histopathological changes over time in a model of surgically-induced osteoarthritic knee joints in rats and to compare the most frequently used subjective semi-quantitative scoring of joint damage with computerized quantitative digital histomorphometry.

Methods: Male skeletally mature Lewis rats (12 weeks of age, n = 10-12 animals/group) were subjected to unilateral transection of the Anterior Cruciate Ligament plus 25% removal of the Medial Meniscus (ACLTpMx model) or sham operated. Three, 7, 28, 56 and 84 days following ACLTpMx the animals were sacrificed and in Hematoxylin-Eosin- and Safranin-O-stained (SO) coronal paraffin sections the degree of joint damage was evaluated by two observers in a blinded fashion using a modified histopathological Mankin score. For quantitative histomorphometry digital images of the joint were analyzed using the digital image analysis software Integrator VIS System Version Nr. 3.0.15.0 (http://www.visipharm.com Denmark). The degree of cartilage destruction and subchondral bone sclerosis was quantified by measuring the following parameters: 1. cartilage surface irregularity, 2. cartilage area, 3. chondrocyte number, 4. area of proteoglycan-containing (SO-stained) cartilage and 5. area of sclerotic subchondral bone.

Results: The histopathological changes (cartilage fibrillation and erosion, chondrocyte loss, proteoglycan depletion and subchondral bone sclerosis) developed rapidly with increasing severity over time. Already 28 days after ACLTpMx moderate to severe signs of OA were observed. Sham-operated animals did not develop significant OA pathology at any time point. The histomorphometric parameters showed a significant correlation with the corresponding Mankin subscores.

Conclusions: The ACLTpMx model of OA in rats shows similar features as human knee OA regarding anatomical location and the specific histopathological morphology. Quantitative digital histomorphometry of cartilage destruction and subchondral bone sclerosis offers a more objective and less time consuming assessment of OA histopathology in this experimental model than classical histopathological scoring, thus facilitating the preclinical pharmacological testing of potential disease-modifying drugs.

129 GAIT ANALYSIS AFTER HYALURONIC ACID INJECTION INTO OSTEOARTHRITIC KNEE JOINTS OF MOUSE

Y. Muramatsu 1, T. Sasho 1, M. Saito 1, S. Yamaguchi 1, N. Ikegawa 1, R. Akagi 1, S. Mukoyama 1, A. Watanabe 2, Y. Wada 2, K. Takahashi 1,2. Dept. of Orthopaedic Surgery, Graduate Sch. of Med., Chiba Univ., Chiba, Japan

Background: Several experimental animal models of osteoarthritis (OA) have been developed to help our understanding of OA. They are especially useful in histological assessments of interventions against OA but behavioral analysis, which is another important aspect of OA feature, has sparsely been performed on them. Gait disturbance results from joint pain associated with OA and gait analysis would be important to evaluate the progression of OA as well as histological evaluation. In the present study, gait analysis was conducted with CatWalk system TM developed for the use of small animals. It is an automated gait analysis system and has been validated as a method to quantify abnormal gait pattern in rat models of arthritic pain. But there has been no comprehensive analysis of its use in mouse model along OA development.
Purpose: To analyze the gait pattern in a mouse model of knee OA using the CatWalk systemTM, and to assess effects of intra-articular injection of hyaluronan (IAI-HA) on gait pattern.

Methods: C57BL/6 mice (9 weeks old, male) were purchased and destabilization of the medial meniscus (DMM) was performed on the left knee joint as OA knee model. In this model, OA progress slowly and it resembles slowly-progressive human OA more closely than other surgical models and allows for suitable evaluation of interventions. The CatWalk systemTM was used for gait analysis. In this system, mice were placed on glass plate runway and allowed to walk freely. The whole run was recorded by a video camera placed below the runway and gait parameters were analyzed according to foot prints by the computer automatically. To quantitatively longitudinal gait changes in DMM model, prior to the surgery, mice (n=5) walked on the runway 10 times for acquiring the baseline data. Then gait analysis was performed at the time point of 4, 8, 12 weeks after the induction of DMM surgery. To assess the effects of IAI-HA to the DMM model, 6 mice underwent DMM surgery, 3 underwent sham surgery where induction of DMM surgery. To assess the effects of IAI-HA to the DMM model, 6 mice underwent DMM surgery, 3 underwent sham surgery where only skin incision and patella luxation was performed. Mice with DMM were randomized to either treatment: (1) HA(800-kDa, ARTZ saline), (2) saline (50 μL of HA or saline injection was done through the patella tendon. Gait analysis was performed for each group of mice at the time point of 3 weeks (just before IAI), 8, 12, and 16 weeks after the surgery.

Results: For each gait parameter, ratio of the affected limb to the contra-lateral limb was evaluated. After the DMM surgery, no significant change was seen in any gait parameter throughout the period of 8 weeks, but significant lower stand phase, single stance phase, duty cycle (percentage of stand phase during the step cycle), swing speed and significant longer duration of swing phase were observed at the time point of 12 weeks. In HA treated experiments, IAI-HA group tended to show better parameters comparing to saline group but they did not reach statistical significance even at 16 weeks.

Conclusion: Gait disturbance was detected at the time of 12 weeks after the DMM surgery. This result corresponded to the previous report that DMM progress OA slower than other surgical models. It was suggested that CatWalk systemTM could be of use to objectively quantify gait disturbance in DMM model. But we could not clarify the effects of IAI-HA on this model by gait analysis using the CatWalk systemTM.

130 APPROACHES TO TRANSCRIPTOME ANALYSIS TO STUDY JOINT REGENERATION IN THE RED-SPOTTED NEWT

C. Knopp 1, T. Borchardt 2, R. Reinhardt 3, S.A. Susanto 1, U. Müller-Ladner 4, M. Geyer 1, R. Dinsen 5, 1Justus-Liebig-Univ. of Giessen, Dept. of Rheumatology and Clinical Immunology, Kerckhoff-Klinik, Bad Nauheim, GERMANY; 2Max-Planck-Inst. for Heart and Lung Res., Bad Nauheim, GERMANY; 3Max-Planck Genom Ctr., Cologne, GERMANY.

Purpose: The adult red-spotted newt Notophthalmus viridescens is able to regenerate damaged knee joints after local injury involving surgically induced defects, collagenase-induced joint instability, and monoiodoacetate-induced cartilage degeneration. The mechanism behind this capacity that is not present in mammals is currently not understood.

Methods: Transcriptome analysis with a custom made microarray from a normalized cDNA library was carried out after surgical and collagenase-induced knee damage in newts. Differentially expressed genes in both instances were validated by qPCR localisation studies. To improve the power of this approach, we have established a new whole new transcriptome library using normalized cDNA from multiple regenerating and normal tissues including heart, extremities, and eyes with the 454 titanium sequencing technique. We are currently performing quantitative transcriptome analysis after knee damage with mRNA sequencing on the illumina platform.

Results: In the initial microarray analysis, a number of gene groups was found deregulated in the course of knee damage repair, most strikingly several matricellular proteins like tenasin. However, several cartilage specific genes like collagen 2 were lacking. The new library is calculated to cover the complete transcriptome with an overlap of 8 fold, annotations are currently being completed.

Conclusions: Conventional array techniques are powerful tools to study differential gene expression. However, the technique is time consuming and has a reduced power compared to the novel techniques that we are currently applying.

131 THE PROGRESSION OF MONOIOODOACETATE-INDUCED ARTHRITIS INVOLES SEQUENTIAL EXPRESSION/SUPPRESSION OF MATRIX ASSOCIATED GENES

B. Rath 1, P. Perera 2, N. Jindal 2, R. Gordon 2, J. Nam 3, S. Agarwal 1. 1Univ. of Aachen, Aachen, GERMANY; 2The Ohio State Univ., Columbus, OH; 3Univ. of California, Riverside, Riverside, CA.

Purpose: Osteoarthritis (OA) is an inflammatory disease with progressive loss of cartilage and bone leading to debilitating joint pain and loss of function. Inflammation is the major cause of cartilage and bone loss. In this report, we examined the gene expression and the signaling networks associated with various stages of cartilage destruction in a rat model of monooiodoacetate-induced arthritis (MIA).

Methods: MIA was induced in the right knee joints of Sprague Dawley female rats (n=60) via intra-articular injection of monooiodoacetate (2 mg/50 μL saline). Saline (50 μL) injected knees served as sham controls. The MIA was temporally monitored macroscopically, microscopically and by μCT (micro-computed tomography) at days 5, 9 and 21 post-MIA induction, and compared to sham controls. Gene Chip analysis (Affimatrix) was utilized to analyze the transcriptome-wide changes in gene expression. The functional networks were generated by Ingenuity Pathways Analysis (IPA), and macroscopic and immunohistochemical findings were correlated with the expression of genes/gene products. Signaling pathways involved in the progression of OA were dissected to focus on salient pathways that drive the cartilage damage during the progression of MIA.

Results: The studies demonstrated that the progression of MIA was progressively damaging to cartilage and underlying bone. In this model of MIA, post-monoiodoacetate injection, Grade 1 damage was observed by day 5, which progressively increased to Grade 2 by day 9, and Grade 3 to 3.5 by day 21. The progression of MIA was accompanied by changes in gene expression, belonging to matrix synthesis/degradation. The maximally upregulated genes in the Grade 1 cartilage damage were genes involved in matrix degradation, those associated with Grade 2 damage were involved in matrix synthesis and degradation, and those associated with Grade 3 to 3.5 damage were involved in matrix synthesis. More importantly, many of these genes were those that have been identified as susceptible genes in human osteoarthritis (OA), such as Asporin, Matrixmetalloproteinase-12 (MMP-12), MMP-19, ADAMTS4, ADAMTS5, CDF5, FZR2 and DIO2.

Conclusions: These findings suggest that sequential regulation of distinct gene clusters involving inhibition of matrix synthesis and induction of matrix degradation may control the progression of cartilage destruction in MIA. In this process, Asporin may act as central node regulating the processes matrix synthesis, whereas inflammatory cytokines regulate matrix degradation.

132 APPA PROVIDES DISEASE MODIFICATION IN PRECLINICAL OSTEOARTHRITIS

S. Glasson 1, A. Bendele 2, N. Larkins 3. 1AK Int'l, Cook, AUSTRALIA; 2Bolder BioPath, Boulder, CO; 3AK Int'l, Barcelona, Spain.

Purpose: APPA, a proprietary combination of apocynin and paeonol, was evaluated for inhibition of cartilage destruction in a well-accepted rat model of osteoarthritis.

Methods: APPA is a synthetic combination of 2 molecules, 4-hydroxy-3-methoxy-acetophenone (apocynin) and 2-hydroxy-4-methoxy-acetophenone (paeonol). Male Lewis rats were anesthetized and aseptic procedures utilized to induce a medial meniscal tear, under an IACUC-approved protocol, APPA was orally administered at 80 mg/kg BID (n=15/group) and animals were euthanized at 3 weeks post surgery. Joints were harvested, fixed in formalin, decalcified, halved in the frontal plane, paraffin