On day 36 after OA induction, the bodyweight of WT mice receiving a high cholesterol diet (n=10) had increased by 21 % compared to WT mice which received a normal diet. Although synovial activation and cartilage destruction was not altered, osteophyte formation was over 33 times higher ($p = 0.0495$) in the medial femur of the high cholesterol group. $LDLR^{-/-}$ mice which received a high cholesterol diet (n=10) expressed high cholesterol levels (500 % higher when compared to WT) within the serum and a significantly increased thickening of the lining layer consisting of macrophages containing high amounts of fat as seen after staining of total knee joints with red oil. Although the OA knee joints of WT mice already showed increased osteophyte formation, the OA knee joints of $LDLR^{-/-}$ developed even higher osteophyte formation (2.7 times higher in the in the lateral tibia; $p = 0.0063$) indicating that the absence of the LDL receptor also induces osteophyte formation under high cholesterol conditions.

Conclusions: LDL receptor deficiency induces osteophyte formation during collagenase-induced osteoarthritis both under low and high cholesterol conditions.

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JOINT HEMORRHAGE ACCELERATED JOINT CONTRACTURE IN IMMOBILIZED KNEE IN RATS

Y. Onoda, Y. Hagiwara, A. Ando, E. Chimoto, H. Suda, K. Sonofuchi, E. Itoi. *Tohoku Univ. Sch. of Med., Sendai, Japan*

Purpose: Joint immobilization is commonly used as a treatment for joint injuries and diseases. However, it also causes unfavorable outcomes such as joint contracture. In our previous reports, joint immobilization caused adhesion and shortening of the joint capsule and restricted range of motion. Joint hemorrhage occurs by intra-articular fractures, ligament ruptures and haemophilia. Some researchers have reported blood-induced joint damage using animal models. Although influence of blood on a synovial membrane (SM) and cartilage matrix was reported, the precise mechanism is still controversial. The purpose of this study was to elucidate capsular changes after single blood injection in immobilized knees in rats.
Methods: **Animals:** The unilateral knee joints of Sprague-Dawley rats aged 12-week old were immobilized at 150° of flexion with a plastic plate and metal screws for various periods (1, 3 days, 1, 2, 4, and 8 weeks). Sham operated rats had holes drilled in the femur and tibia with screws, but the plate was not inserted. After the operation, the rats were divided into three groups: Immobilized-blood injection (Im-B) group, Immobilized-normal saline injection (Im-NS) group, and Sham-blood injection (Sm-B) group. Fifty μl of autologous blood were administered intra-articularly for the Im-B and Sm-B groups just after the surgery. The same amount of normal saline was administered for the Im-NS group.
Histology & Immunohistochemistry (IHC): Paraffin embedded 5-μm thick sagittal sections in the medial midcondylar region of the knee were made. The sections were stained with Elastica-Masson to observe morphological changes of the SM and the capsule, and with Perls' Prussian blue to visualize iron deposition in the SM and capsule. The expression patterns of CD68, TGFβ1, and collagen types I and III in the capsule were evaluated by IHC.
Scanning acoustic microscope (SAM): In general, sound speed is in proportion to the square route of Young's elastic modulus. SAM can measure sound speed of tissues on slide glass in situ. We set the region of interests and their average sound speed was calculated with a gray scale SAM images with image analysis software.
Results: Histology & IHC: Absorption of the injected blood was delayed and made severe adhesions in the Im-B group (Fig. 1A-C). Shortening of SM was observed in the capsules due to adhesion of the opposing capsule, the articular cartilage, and the meniscus. The length of posterior SM in the Im-B group was significantly shorter than that of the other groups from 1 to 4 weeks (Fig.1D and E). The iron deposition in the capsule was observed in the Im-B and Sm-B groups (Fig. 2A-C). Strong immunoreactivity of CD68 and TGF-β1 were observed in the adhesion areas in the Im-B group (Fig.2D- I). However, the staining intensity of collagen types I and III did not change in the Im-B group compared to the other groups.

SAM: The low sound speed areas decreased and high sound speed areas increased in the posterior capsule in the immobilized group (Im-B and Im-NS) (Fig.3A- C). The sound speed of posterior capsule in the Im-B group was significantly higher than that in the Sm-B group (Fig. 3D and E).
Conclusions: These data indicated that joint immobilization and blood injections caused irreversible capsular changes. Joint hemorrhage is a risk factor for joint contracture, and drainage of the blood or short immobilization periods might be a good strategy to avoid joint contracture.

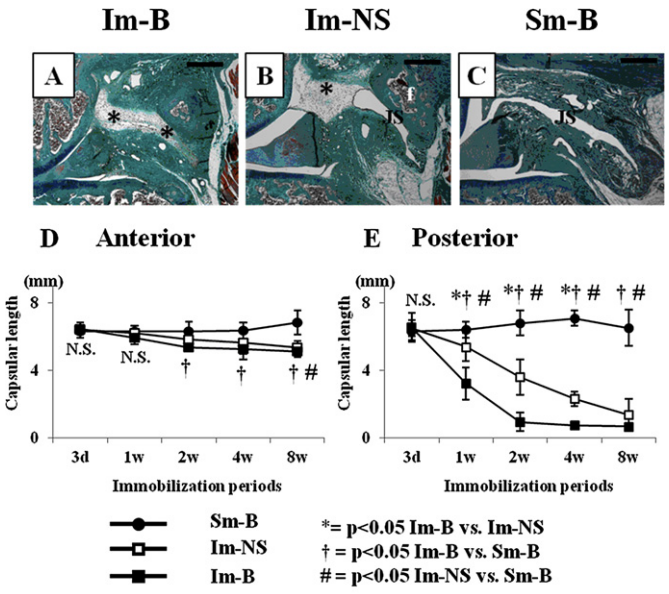

Figure 1. A-C: Posterior Capsule at 2 week. Scale bars = 500 μm.
JS: Joint space, *Arrowhead*: Positive cells, *Asterisks*: Adhesion area.
D and E: Length of the synovial membrane (SM).
D: Total Anterior length, E: Total posterior length.

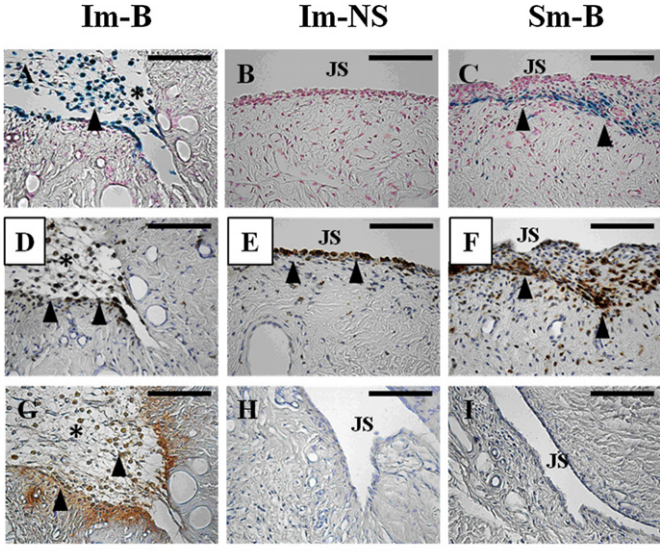

Figure 2. Posterior Capsule at 1 week. Scale bars = 100 μm.
A-C: Perls' Prussian blue, D-F: CD68, G-I: TGF-β1
JS: Joint space, *Arrowhead*: Positive cells, *Asterisks*: Adhesion area.

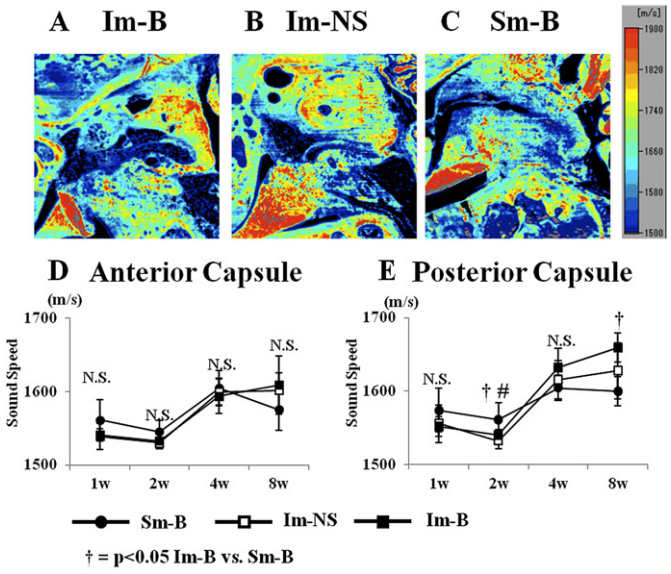

Figure 3. A- C: Posterior Capsule at 8 week.
Low sound speed area: Black to blue, High sound speed areas: Yellow to red.
D and E: The sound speed of the capsule.

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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 and the TGFα pathway as viable candidates 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.

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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 osteoarthritis of the knee joint 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.visiopharm.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.

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GAIT ANALYSIS AFTER HYALURONIC ACID INJECTION INTO OSTEOARTHRITC 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]. [1] *Dept. of Orthopaedic Surgery,Graduate Sch. of Med.,Chiba Univ., Chiba, JAPAN;* [2] *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.