received 3 times per week 25 IMJ of 0.2 ml isotonic solution, CS group - received 3 times per week 25 IMJ of 0.2 ml 10% solution CS. After euthanasia tissues were fixed for 24 hours in 10% neutral buffered formaldehyde. Kidney, heart and liver were investigated by histological and histochemical methods. The material was studied by blind method.

Results: At the time of sacrifice, 12 animals survived in placebo group, in the CS group - 15 males and 10 females. The alive animals were decapitated. In placebo group fibrocartilage articular surface was destroyed and remodelled. Tide mark was absent. Acid glycosaminoglycans (GAG) depletion and collagen network fragmentation have been estimated. Severe vasculitis in all organs were found. There were severe nephritis, myocarditis and hepatitis too. In the CS group cartilage was uniform, with normal thickness and layer differentiation. Cartilage repair processes have been estimated as follows - the number of double-nuclear chondrocytes increased and accumulated in groups. GAG increased too. Collagen structure re-established. Tide mark was safe. Dystrophy and mild vasculitis in heart and liver, severe alteration in kidney were found. There was significant difference between the group results (p<0.05).

Conclusion: Not only cartilage, but organic lesions, such as vasculitis, nephritis, myocarditis and hepatitis are influenced after chondromodulators' treatment of MRL/l mice. These results provide some insight into the anti-inflammatory mechanism of CS action.

121 FOLLISTATIN ALLEVIATES SYNOVITIS AND ARTICULAR CARTILAGE DEGRADATION INDUCED BY CARRAGEenan IN MICE


Purpose: Osteoarthritis (OA), a chronic degenerative joint disorder characterized by articular cartilage destruction and osteophyte formation, is prevalent in our society as a major cause of disability. Molecular pathogenesis of OA is not fully understood, it is considered that acute joint inflammation plays significant roles in the onset of OA. This idea is supported by various animal experiments, since articular cartilage degradation is induced by the intra-articular injection of carrageenan or zymosan. Previously we showed that intra-articular injection of recombinant human BMP7 (rhBMP7) inhibited cartilage degradation induced by zymosan partly through the inhibition of inflammatory cytokine expression such as IL-1 beta in the joint. From these data we hypothesized that TGF-beta/BMP signal maintains joint homeostasis by controlling inflammatory status. Here we report that follistatin, an endogenous inhibitor for Activin which belongs to TGF-beta/BMP family and works as a proinflammatory cytokine, effectively alleviates synovitis and articular cartilage degradation induced by the intra-articular injection of carrageenan in mice.

Methods: This study was approved and conducted in accordance with the guideline of the animal committee of Tokyo Medical and Dental University. Male C57BL/6j mice (12 weeks old) were purchased from ORIENTAL YEAST co.,Ltd (Tokyo, Japan). They were housed under a 12-h light-dark cycle and allowed food and water ad libitum. Twelve mice were randomly divided into two groups (n=6/group). Mice were anesthetized by the inhalation of 5% isoflurane in oxygen. Under deep anesthesia, a solution of 30g/L lama-carrageenan (Sigma-Aldrich) in 5µl saline was injected into the left knee joint through the lateral margin of the patella tendon. Recombinant mouse follistatin (25ng in 5µl in physiological saline, Sigma-Aldrich) was injected into the left knee at 30 minutes before carrageenan challenge. Mice were maintained in cage ad libitum for 3 days after the challenge. Knee joints were dissected, fixed in 4% paraformaldehyde, decalcified, embedded, and 5µm sagittal sections were prepared for histology. Integrity of articular cartilage and synovium was assessed by Hematoxylin and Essin staining. To assess articular cartilage damage, three sections (apart from 150µm respectively) were stained with Safranin O and 400 µm in width of articular cartilage between anterior and posterior edge of medial meniscus was contoured into 3 areas according to the dyeability: Grade I; intact cartilage, Grade II; mildly denatured cartilage with reduced safranin O staining, and Grade III; severely denatured cartilage with no Safranin O staining. Each area was measured using Zeiss Axios Vision Image Analysis system. Kruskal-Wallis test followed by Tukey-Kramer methods was used for statistical analysis.

Results: Image analyses indicated that dyeability of articular cartilage by Safranin O was significantly reduced by the single intra-articular injection of carrageenan at 3 days (Grade I: 21.4%, Grade II: 33.8%, Grade III: 44.8%), although we did not observe any obvious alteration in the articular surface structure at this stage. In contrast, the loss of dyeability after carrageenan injection was significantly improved by the pre-injection of follistatin (Grade I: 26.4%, Grade II: 73.6%, Grade III: 0%, p<0.05). Hematoxylin and Essin staining showed that the cellularity of synovial tissue is greatly increased in carrageenan-injected mice. In contrast, these inflammatory responses were greatly alleviated by the pre-injection of follistatin.

Conclusions: Carrageenan-induced arthritis is a well-established experimental model to investigate inflammation-mediated articular cartilage degradation in rodents. Here we report that follistatin effectively inhibits the loss of Safranin O dyeability of articular cartilage induced by carrageenan. Our data strongly suggest that the Activin signal pathway is involved in the process of joint inflammation and proteoglycan loss in the cartilage matrix. We believe that our experimental system will be of great use for analyzing the molecular events underlying the onset of OA.

122 NEW EVIDENCE LINKING THE IN VIVO IMPLICATION OF 4-HYDROXYNONENAL IN THE OSTEOARTHRITIS PATHOGENESIS.

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Purpose: To demonstrate the in vivo involvement of 4-hydroxynonenal (HNE), a major aldehyde derived from lipid peroxidation of n-6 polyunsaturated fatty acids, in the osteoarthritis (OA) pathogenesis.

Methods: Protocol 1 - OA was induced by anterior cruciate ligament transection of the right knee in crossbred dogs. There were two experimental groups (n=4 dogs/group): placebo and carnosine (an HNE-trapping drug, 50 mg/kg/day), given orally for 8 weeks. Protocol 2 - Vehicle or pathophysiological dose of HNE (100 µM) were injected weekly into the right knee joint of crossbred dogs (n=4 dogs/group) for the entire duration of the study (16 weeks). We conducted macroscopic and histomorphological analyses of cartilage of the femoral condyles and/or tibial plates. We also conducted immunohistochemical analyses in cartilage explants for the following antigens: HNE, agrecanase-2, and matrix metalloproteinase -13 (MMP-13).

Results: In the protocol 1, treatment with carnosine reduced the severity and histopathological score of OA cartilage lesions as well as the levels of HNE, MMP-13 and agrecanase-2 in cartilage explants. In the protocol 2, the intraarticular injection of HNE induced cartilage lesions on the tibial plateaus and femoral condyles with prominent osteophytes on lateral condyles. The expression of both MMP-13 and agrecanase-2 increased in cartilage explants from HNE-treated dogs.

Conclusions: This is the first in vivo study to demonstrate the pathophysiological role of HNE in OA. The fact that carnosine abolishes HNE production and a number of factors known to be involved in OA pathogenesis renders it a clinically valuable agent in the prevention of this disease.

123 A COLLAGEN-PLATELET COMPOSITE TO STIMULATE HEALING AFTER ACL SURGERY ALSO MINIMIZES CARTILAGE DAMAGE IN THE ACL INJURED KNEE


Purpose: ACL injury is a risk factor for early post-traumatic osteoarthritis (PTOA), and the gold standard of treatment, ACL reconstruction, does not reduce this risk. The mechanism of PTOA in the ACL injured joint is likely due both to the initial inflammation and ongoing subtle mechanical instability. Recently, intra-articular implantation of a collagen-platelet composite (CPC) during surgery in animal models has been shown to improve healing following bio-enhanced ACL repair or ACL reconstruction procedures, though the impact of the CPC on articular cartilage remains unknown. We hypothesize that cartilage integrity following bio-enhanced
ACL repair (BE-repair) and bio-enhanced ACL reconstruction (BE-ACLR) is improved when compared to traditional ACL reconstruction (ACLR) or ACL transection with no treatment (ACLT).

**Methods:** With IACUC approval, 31 adolescent minipigs underwent surgical ACL transection in one knee followed by BE-repair (n=8), BE-ACLR (n=8), and no treatment (ACLT; n=7). After 12 months of healing, the articulating surfaces of the surgically treated and contralateral ACL intact knees were macroscopically graded following application of India ink using a five point scale (0=no changes; 1=interface with color changes; 2=surface fibrillation; 3=exposed bone10%). The surface areas of all lesions were determined using calipers and an ellipsoidal fit. A mixed linear model was used to make comparisons between treatments (BE-repair, BE-ACLR, ACLR, and ACLT) and compartments (medial femoral condyle, lateral femoral condyle, medial tibial plateau, and lateral tibial plateau). Similar analyses were performed to compare the lesion areas within each animal. All statistical analyses were done on the difference between the surgical and contralateral ACL-intact knee within each animal.

**Results:** We found significant mean differences in cartilage scores between treatments (p=0.05) and compartments (p=0.01). The mean difference ± confidence interval for BE-repair, BE-ACLR, ACLR and ACLT (pooled across compartments) were 0.24±0.193, 0.16±0.241, 0.48±0.181, and 0.66±0.392, respectively. Only the knees treated with BE-ACLR did not have increased chondral injury on the surgical side. For the lesion area measurements, the treatment effect was statistically significant in the medial femoral condyle (p=0.012). The mean difference ± confidence interval between the surgical and contralateral ACL-intact knees for BE-repair, BE-ACLR, ACLR and ACLT were 5±17.8mm², -19.1±23.1mm², 40±31.9mm², and 57±51.5mm², respectively. Both the BE-repair and BE-ACLR procedures resulted in mean differences between the operative and non-operative side that were not significantly different from zero. It should also be noted that there were no lesions in either the surgical or contralateral ACL intact knee in the lateral femoral condyle or medial tibial plateau for any animal undergoing BE-repair.

**Conclusions:** ACL transection and ACL reconstruction both resulted in increased chondral damage of the knee at one year after surgery as noted in humans. In contrast, treatment of the ACL transection with either bio-enhanced repair with CPC or ACL reconstruction augmented with CPC prevented this increased chondral damage. These data suggest that the intra-articular application of CPC may be chondroprotective.

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**124 CHRONICITY PRODUCES DIFFERENTIAL ALTERATIONS IN PAIN BEHAVIORS IN MURINE OSTEOARTHRITIS**

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**Purpose:** Osteoarthritis pain is a growing problem due to an expanding elderly population and lack of safe and effective therapies. Understanding the origins and pathogenesis of chronic arthritis pain is important for developing effective therapies. Osteoarthritis pain does not correlate well with radiographic severity, and loss of function in patients with osteoarthritis may result from pain, from altered biomechanics or from toxicity of analgesics. In order to better understand the relationship between osteoarthritis, pain, and function, we measured different types of pain behaviors in mice with osteoarthritis as a function of age, duration of arthritis and in response to different analgesic treatments.

**Methods:** Collagenase (10 IU) in 10 μl was given by intra-articular (IA) injection into the left knee of 4-week-old C57Bl6 male mice. This produced osteoarthritis that was identifiable histologically after 4 weeks. Arthritic mice were compared to uninjected naïve mice of the same age at 4 weeks and 6 weeks after collagenase injection and to arthritic mice treated with IA analgesics. Analgesics tested were IA morphine sulfate (0.7 mg/kg in 5 μl) or IA Botulinum toxin type A (BoNT/A) (0.02 IU given 3 d before testing). Pain behavior measures included evoked pain response to repetitive firm palpation of the knee for 1 minute, voluntary, spontaneous nocturnal wheel-running, mechanical withdrawal thresholds by Von Frey filament testing and digitized video gait analysis using DigiGait10M (Mouse Specifics, Inc, Quincy, MA). The nonarthritic knee was the internal nonpainful control.

**Results:** At both 4 and 6 weeks after collagenase injection, evoked pain responses in arthritic knees were increased, but this response was 65% greater at 4 weeks than at 6 weeks. Arthritis caused an increased swing/stride ratio measured by gait analysis and increased mechanical allodynia in the arthritic limb by Von Frey filament testing at both 4 and 6 weeks. Von Frey testing in the normal right limb revealed allodynia at 4 weeks but an increased pain threshold at 6 weeks. Spontaneous nocturnal wheel-running was reduced in both the 4 and 6 week arthritic groups, as well as in 6 week naïve mice. Both BoNT/A and Morphine were effective analgesics at 4 and 6 weeks as measured by evoked pain but did not normalize gait function at either time point. Only morphine normalized the threshold for mechanical allodynia to Von Frey filament testing at 4 weeks, but IA BoNT/A was more effective at 6 weeks.

**Conclusions:** IA injection of collagenase in mouse knees produces arthritis pain that can be measured by various methods at 4 weeks and persists to 6 weeks. Both opioids and BoNT/A given IA are effective analgesics when pain is measured by evoked pain behavior. Changes in functional measures such as gait analysis do reflect the development of arthritis pain but are not clearly normalized by analgesia and may be due not only to pain but to biomechanical changes in the joints. Chronic osteoarthritis pain produces mechanical allodynia, one indication of peripheral sensitization. Mechanical allodynia may be decreased by opioids if arthritis pain is not longstanding but IA BoNT/A may be more effective for sensitization due to arthritis that is more chronic. More work needs to be done to determine which pain behaviors best measure chronic pain in murine arthritis and are sensitive for detecting analgesia in order to use preclinical models for testing potential new analgesics.

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**125 LDL RECEPTOR DEFICIENCY RESULTS IN INCREASED OSTEOPHYTE FORMATION DURING EXPERIMENTAL OSTEOARTHRITIS BOTH UNDER LOW AND HIGH CHOLESTEROL CONDITIONS**


**Purpose:** Synovial macrophages have previously shown to be involved in joint destruction during experimental collagenase-induced osteoarthritis (OA). The low density lipoprotein (LDL) receptor expressed by synovial macrophages is involved in transport of cholesterol, carrying lipoprotein particles into cells, thereby regulating cholesterol homeostasis. In the present study we investigated whether the LDL receptor is involved in joint destruction during experimental osteoarthritis both under normal and high cholesterol conditions.

**Material and Methods:** LDL receptor deficient (LDLR/-) mice and their wild type (WT) controls received either a high cholesterol or control diet for 120 days. Experimental osteoarthritis was induced by injection of collagenase into the mice’s knee joints on day 84 and 86. Paraffin sections of total knee joints were stained with safranin-o or haematoxylin-eosin to determine OA development. Synovial activation (thickening of the lining layer) was measured using an arbitrary scale from 0 to 3. Cartilage destruction was determined in four cartilage surfaces (lateral and medial femur and tibia) using the OA score (with a maximum of 30 per knee joint) developed by Pritzker et al (2006) and adapted by our lab for mice. Size of osteophyte formation was measured on the edges of the femur/tibia area using image analysis. Results are depicted as mean ± SD.

**Results:** On day 36 after induction of collagenase-induced OA, WT mice which received a normal diet (n=10) developed moderate synovial activation (1.4 ± 0.6), cartilage destruction (6.1 ± 2.6) and osteophyte formation (32.4 ± 25.4). In LDLR/- mice (n=10) no significant differences were found on synovial activation or cartilage destruction when compared to WT controls. In contrast, mean osteophyte formation was tremendously increased by 345 % suggesting that the absence of the LDL receptor induces osteophyte formation in the OA knee joint.