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A RABBIT KNEE MODEL OF CONTROLLED JOINT INSTABILITY

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Purpose: Joint instability associated with an ACL injury is a well-recognized risk factor that leads to post-traumatic OA in the human knee. Therefore, ACL transection (ACLT) has been employed in several animal models to investigate post-traumatic OA. In ACLT rabbit models, advanced OA predictably develops within 8 weeks of transection. Unfortunately, the severity and rapidity of OA that develops in the rabbit ACLT model is not optimal for piloting therapeutic interventions, or for investigating interaction of instability with other pathogenic factors. The goal of this study was to develop a rabbit model of controlled knee instability in which OA develops reproducibly at a level more amenable to therapeutic interventions. The hypothesis was that, by inducing a different level of instability in rabbit knees with partial (rather than full) and total ACLT, reproducible, sub-critical level cartilage degeneration would occur.

Methods: With institutional approval, sixty New Zealand White rabbits received either total ACLT (n = 20), partial (medial half) ACLT (n = 20), or sham surgery (control, n = 20) on their left knees. Halves of animals were studied for 8 weeks after surgery, and the other halves for 16 weeks. At the end of study period, their knees were subjected to a loading test in which AP stability was quantified in terms of anterior drawer stiffness and neutral-zone length. Conditions of the ACL and menisci were inspected macroscopically. The joints were then harvested for histo-morphological evaluation. The primary weight-bearing regions of the femoral and tibial surfaces in both medial and lateral compartments were rated individually on the Histological Histochemical Grading Scale (HHGS, 14 points max). The average of the four individual surface scores for each joint was defined as the whole-joint score.

Results: AP stability was impaired by both types of ACLT, as evidenced by decrease of anterior drawer stiffness and increase of neutral zone length (Figure 1), with the effect higher with total ACLT. All partially transected ACLs remained unruptured. In the total ACLT knees, medial meniscus tear was frequently observed (14/20 joints). The whole-joint HHGS scores in both types of ACLT knees were higher than control, with the effect higher with total ACLT (p < 0.01, Figure 2). The medial tibial surface was most frequently affected, though degeneration was mild (3 ≤ HHGS < 6) in every case. In the medial femoral surface at 16 weeks, severe degeneration (HHGS ≥ 9) was observed in 4/10 knees. Statistical differences between study periods were not detected for any surface. There was a trend that higher degrees of degeneration occurred with higher degrees of instability (Figure 3).

Conclusions: The medial half ACLT introduced modest instabil-

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OSTEOARTHRITIS-LIKE CHANGES IN SPONDYLOEPITHESEAL DYSPLASIA CONGENITA (SEDC) MUTANT MICE

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Purpose: A naturally occurring missense mutation in the CoI2a1 gene (which normally encodes for types II and XI procollagen) has recently been identified in the mouse. The human SEDC mutation of the CoI2a1 gene has been shown to cause mild dwarfism and premature degeneration of the articular cartilage. The present study has a three-fold purpose: (1) to characterize the articular cartilage (AC) in sedc mice specifically by identifying cellular changes over time and localizing the major structural proteins present in the extracellular matrix (ECM); (2) to determine the mechanism involved in the degeneration of AC; (3) to establish the sedc mouse as a new murine model for investigating the mechanism of OA.

Methods: We compared sedc homozygous mutant mice with age-matched, wild-type (WT, +/+ ) controls at 2, 6, 9, and 12 months of age. We performed histological, ultrastructural, and immunohistochemical (IHC) analyses to determine the changes that occur in the articular cartilage of the knee joint of sedc mice. Histological sections were stained with Hematoxylin/Eosin and Safranin O/Fast Green stains to show the presence and localization of proteoglycans and collagen. Ultrastructurally we looked for cytological changes pertaining to the secretion of proteins and the development of the ECM. IHC analysis was performed using the 1C10 antibody against the structural protein type II collagen. Additional IHC analysis was done to determine the mechanism of degradation using antibodies against the collagen-degrading enzyme MMP-13 and the aggrecan degradation epitope VDIPEN.

Results: Upon histological analysis, 2 month sedc homozygotes demonstrated increased articular cartilage thickness and disorganization of chondrocytes compared with controls. Disorganization was accompanied by an abnormally enlarged pericellular space that surrounded each chondrocyte, adding to a decreased amount of ECM compared to WT mice, which exhibited normal tissue architecture. Sedc homozygotes also exhibited fissuring in the articular cartilage as early as 6 months of age whereas the WT animals did not. Ultrastructurally we found that the enlarged pericellular space contained non-fibrillar material, thereby decreasing the amount of fibril formation in the ECM. With IHC analysis of the sedc homozygote mouse the pericellular space surrounding the chondrocytes stained positive for type II collagen, whereas staining in the intercellular space for type II collagen was less intense relative to control. At this time IHC performed with antibodies against MMP-13 and VDIPEN were presently being analyzed.

Conclusions: These data suggest that sedc mutant mice are incapable of correctly producing proteins integral in the formation of ECM which may be due to incorrect folding of type II collagen. The inability of proteins to polymerize and the lack of fibril formation would decrease the integrity of the ECM. This defective ECM may be involved in the up-regulation of destructive enzymes, such as MMP-13, which would therefore lead to the premature degeneration of AC. Thus, the sedc mouse presents itself as a viable murine model for the study of OA.

Figure 1. Effects of ACL transection on joint laxity measures.
Metabotropic glutamate receptors (mGluRs) are widely expressed in the CNS, where they function to modulate neuronal excitability and synaptic transmission. Recent anatomical and behavioral data show the expression of G-protein coupled mGluRs in the periphery on nociceptive primary afferent nerve terminals, and provide evidence for a functional role of peripheral mGluRs in inflammatory pain. Studies in animal models of inflammatory pain demonstrate that central and peripheral group I mGluRs are involved in nociceptive transmission in the normal and the inflamed state but group II, III mGluRs are not well established that modulation of peripheral mGluRs reduces pain behaviors and nociceptor activity in arthritis. In the present study, we examined whether the group II, III mGluRs were involved in maintenance of behavioral signs of non-evoked pain and secondary mechanical hyperalgesia induced by knee joint inflammation.

**Methods:** Complete Freund adjuvant (CFA, 100 μl) and MIA (4 mg/50 μl) were injected into the knee joint space to induce arthritis in male Sprague-Dawley rats under light enflurane anesthesia. Group II mGluR agonist, APDC (10, 500 μM/50 μl), group II mGluR antagonist, LY341495 (200, 500 μM/50 μl), group III mGluRs agonist, L-AP4 (10, 500 μM/50 μl) and group II/III mGluRs antagonist, CPPG (200 μM/50 μl) were intra-articularly (i.a.) injected 5 days after induction of arthritis and behavioral tests for non-evoked pain and mechanical hyperalgesia were conducted for 120 min.

**Results:** APDC (100 μM) partially reversed the reduction of weight load whereas withdrawal threshold significantly increased both 10 and 100 μM of APDC. L-AP4 showed an antinociceptive effect on weight bearing (500 μM) and secondary mechanical hyperalgesia (10, 500 μM). However, LY341495 and CPPG did not reduce the change of weight load and withdrawal threshold to mechanical stimuli in maintenance of acute knee joint inflammation in rats.

**Conclusions:** The present study demonstrated that peripheral group II, III mGluRs were involved in maintenance of chronic arthritic pain induced by CFA and MIA in rats. It is suggested that peripheral group II, III mGluRs play a role in inflammatory pain including primary and secondary hyperalgesia.

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RELATIONSHIP BETWEEN CANINE STIFLE STRUCTURAL DAMAGES AND FUNCTIONAL IMPAIRMENT IN EXPERIMENTAL OSTEOPOROSIS: PODOBAROMETRIC GAIT ANALYSIS COUPLED WITH 1.5 TESLA MAGNETIC RESONANCE IMAGING

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Purpose: Lameness is a cardinal feature of osteoarthritis (OA) and reflects painful functional impairment. The impact of OA joint structural damages on the dog’s gait is to date poorly documented. To gain insight to the structure-function relationship of the impaired joint, we explored the relationships between joint structural damages induced in a dog model of OA, as assessed with a high-field (1.5T) magnetic resonance imaging (MRI) and limb function over a 26-week period.

**Methods:** In a prospective experimental study, OA was surgically induced by transection of the right cranial cruciate ligament in five dogs. Peak vertical force (PVF), ground contact area (GCA) and MRI were acquired at baseline (before), as well as 4, 8, and 26 weeks post-surgery on the dog’s right stifle. Osteophytosis, joint effusion, meniscal tears and degenerative changes, cartilage defect as well as subchondral bone marrow lesions (BML) were scored using semi-quantitative ordinal scales. Quantitative changes in cartilage volume were determined from computerized reconstruction. Statistical exploratory analysis used Spearman correlation tests and repeated-measures analysis of variance at the 0.05 α-threshold.

**Results:** As expected, limb impairment (lower PVF and GCA) and OA joint damages (MRI) were significantly induced in this model. More specifically, severe impairment (week 4) was followed by incomplete remission (evolution over the week 4 to 26 period). During the remission phase, the increase in PVF correlated significantly with less severe cartilage defect (p=0.004, rho=0.97), osteophytosis (p=0.037, rho=0.90), joint effusion (p=0.013, rho=0.95) and BML (p=0.001, rho=0.99) while being slightly associ-