Results: Following ACL section, limb impairment rapidly developed in all dogs, with PWV 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 PWV 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 insignificant effect on limb impairment and recovery. Both BML-SPGR and cartilage defect adversely affected the recovery in PWV, with mutually independent effects. Similar to In_PWV, 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 impairment were related mostly to BML and joint effusion. Our data from dogs with experimental OA confirm the role of BML and joint effusion on limb function. On one hand, BML and cartilage defect hinder the recovery of PWV. 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.

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

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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 unimpeded. 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 transaction with partial medial meniscectomy (ACLT+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 ACLT+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 Bonferroni 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 stepcycle 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 sensitisation 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±586pmol/g in the ACLT+pMMx and MIA models.
respectively (controls: 2541±293 pmol/g) (p<0.05). Although substance P levels in the ACLT+pMMx (160±39 pmol/g) and the MIA model (226±67 pmol/g) were elevated when compared to the control group (111±49 pmol/g), this difference was not statistically significant. However, dynorphin concentrations were significantly decreased with concentrations of 370±70 and 418±107 in the ACLT+pMMx and MIA models respectively (controls: 779±111 pmol/g) (p<0.05). Histological evaluation revealed severe cartilage loss in the MIA model and comparatively minor changes in the ACLT+pMMx model, confirming articular lesions in both models.

Conclusions: Clearer behavioural nociceptive responses related to gait parameters of the osteoarthritic limb were seen with the MIA model suggesting that it may be a better model for the evaluation of therapeutic strategies for joint pain palliation when compared to the ACLT+pMMx model, with respect to the length of the study.

091
PKCζ DELETION DECREASES OSTEOARTHRITIS FOLLOWING SURGICAL INSTABILITY

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Purpose: Protein Kinase C zeta (PKCζ) is an atypical PKC that is up-regulated in human osteoarthritis (OA) and has a role in aggrecanase induction and proteoglycan release. This study was designed to compare the severity of osteoarthritides (OA) in wild-type (WT) and PKCζ knock out (KO) mice following challenge in a surgical-instability model.

Methods: All animal studies were performed with IACUC approvals. The PKCζ KO was generated in-house on a 129SvEv background with deletion of exon 7 resulting in a frameshift stop and elimination of both PKCζ and PKMζ expression. PKCζ KO and sibling WT male mice (22 per group) underwent surgical destabilization of the medial meniscus (DMM) when 10 weeks of age, and were group housed until sacrificed 8 weeks later. Following euthanasia, right knees were collected for histopathologic evaluation and were group housed until sacrificed 8 weeks later. Following euthanasia, right knees were collected for histopathologic evaluation of cartilage degeneration. Safranin-O stained paraffin sections were examined at 80μm intervals through the joint to generate both summed and maximal scores from all 4 quadrants of the femoro-tibial joint.

Results: The PKCζ KO mice were grossly normal with no fertility issues. Severe cartilage degeneration was observed in the sibling WT at 8-weeks following DMM. In contrast, the PKCζ KO had only mild to moderate cartilage degeneration. The cartilage degeneration scores revealed very significant decreases in summed and maximal scores in the PKCζ KO, when compared to sibling WT.

Conclusions: Previous studies have found that PKCζ expression is transcriptionally up-regulated in human OA cartilage compared to normal cartilage, is rapidly phosphorylated following IL-1 stimulation, and PKCζ inhibitors can block aggrecanase expression and proteoglycan release from cartilage explants. This study demonstrated that deletion of the catalytic domain of PKCζ (present in both PKCζ and PKMζ) provides significant protection in the DMM model in the mouse. Previous DMM studies (not published) have shown a lack of protection with a different PKCζ KO, which maintained PKMζ expression. These findings indicate the importance of the catalytic domain of PKCζ and the potential of inhibitors selectively directed to this region to provide disease-modification for the treatment of OA.

092
VALIDATION OF AN EXPERIMENTAL DEVICE SIMULATING THE STANCE PHASE OF A CANINE HINDLIMB AT TROT IN THE CRANIAL CRUCIATE DEFICIENT STIFLE: AN IN VITRO KINEMATICS STUDY

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Purpose: Animal models are frequently used in medical research. The use of the Pond-Nuki model is well established in the field of Osteoarthritis (OA). In the past years, the use of 3D kinematics has gained in popularity because it provides objective means of assessing the function of joints/limbs. To this date, most in vitro studies analyzing kinematics of the canine stifles were made in 2D under low static loads. To the authors’ knowledge, this model is the first quasi-dynamic weight bearing in vitro model of 3D kinematics of the canine cranial cruciate ligament deficient stifles.

Goal: Our goal was to use a validated experimental device simulating a quasi-dynamic model of the stance phase at trot of the canine hind limb under near physiologic conditions in the cranial cruciate ligament deficient stifles (CCLDS).

Hypotheses: 1) Kinematics generated by the device is representative of reported in vivo 3D kinematics in dogs with CCLDS; 2) Peak vertical forces generated by the loaded limbs in the device are comparable to those recorded in the literature for a trotting dog during the stance phase.

Methods: Six normal paired hind limbs were harvested from 3 adult large breed dogs euthanatized for reasons unrelated to this study. The dogs were similar in age, size and body weight (range, 29.5-31 kg). The limbs were prepared and mounted on a previously validated experimental device. Each limb was submitted to vertical loading (9 kg). The gait was simulated with a computerized sequence using a linear actuator and a rotational motor. The stance phase of the gait at trot was simulated three times on each hind limb. Then, with the limbs still mounted in the device, the cranial cruciate ligament (CCL) was completely transected through a small medial arthrotomy which was closed using a simple continuous pattern. Kinematics of the tibia and femur was measured in the 2 situations (intact and CCLDS) with an optoelectronic system. Vertical ground reaction forces were measured with a 2.5 kN axial/torsion force transducer. Amplitude of motion and peak ground reaction forces as well as the general shape of kinematics curves were also compared with in vivo curves described in the literature.

Results: The comparison of the six average curves of motion collected on the tested stifles to those from in vivo trials reveals similar patterns. Data recorded during in vitro simulations in CCLDS highlighted the following changes between intact and CCLDS: an increase in flexion (4°), abduction (2°), internal rotation (3°), cranial translation (4mm), medial translation (1.5mm) and proximal displacement (3mm) of the tibia were recorded. These changes are comparable to those reported in vivo. However, the amplitude of changes is slightly greater on in vivo curves. Peak vertical forces measured in the device (138±2N) were also similar to in vivo trials reported in the literature.

Conclusions: Results show that the device generates reliable motion on a loaded limb which is representative of the in vivo 3D kinematics in CCLDS reported in the literature. This model could be used to evaluate the impact of OA on kinematics in the Pond-Nuki model. Furthermore, this model could be used ex vivo to evaluate the effect of different therapeutic modalities of OA. Finally and of utmost importance, this model could be adapted and used with the human knee.