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DESTABILIZATION OF THE MEDIAL MENISCUS AS A MODEL FOR THE STUDY OF PAIN PATHWAYS ASSOCIATED WITH DEVELOPMENT OF MURINE OSTEOARTHRITIS

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Purpose: Pain is the major symptom in osteoarthritis (OA) and one of the leading causes of impaired mobility in the elderly. There is no simple correlation between pathological changes in the joint and pain severity. No animal models adequately describe the correlation between joint structure and symptoms, and very few have properly examined mechanisms of OA pain generation. We recently reported that, following DMM in the right knee, C57BL/6 mice develop rapid-onset progressive mechanical allodynia in the ipsilateral hindpaw only, as early as 2 weeks and developing over 8 weeks post DMM surgery. *Adamts5* knockout (KO) mice, which do not develop OA-like structural changes after DMM, did not develop allodynia. Our long-term goal is to quantitatively measure pain and dissect molecular pathways involved in pain generation in a mouse model of OA. We chose destabilization of the medial meniscus (DMM) in C57BL/6 mice because, unlike other rodent OA models, the joint pathology in this model is slowly progressive over 16 weeks and thus optimally suited for studying pain at different stages of disease.
Methods: DMM surgery was performed in the right knee of 10-week old male C57BL/6 mice. At different time points after surgery (2, 4, 8, 12 and 16 weeks), pain was assessed in DMM operated mice, in sham controls and in age-matched naïve controls. Pain-dependent measures included 1) von Frey analysis to measure mechanical allodynia; and 2) behavioral monitoring with Laboras™ equipment, which quantifies activity using pattern recognition software, including total distance traveled over a specified time period. Concurrently, innervating dorsal root ganglia (DRG) L2-L5 were harvested from both the operated and the non-operated side at different time points after surgery for RT-PCR analysis. Knee joints were collected for histopathology or India ink staining.
Results: In the current studies, we found that the unilateral mechanical allodynia subsided 8-16 weeks after DMM surgery. Total distance traveled over a specified period was relatively constant over the first 8 weeks after surgery, but decreased from 12 weeks after surgery. RT-PCR analysis of innervating DRG at different time points (4, 8, 16 weeks) following DMM revealed significantly increased levels of mRNA (compared to naïve age-matched controls) for the neurotrophic factors, NGF (nerve growth factor) and GDNF (glial cell-derived neurotrophic factor), and for the chemokine, MCP-1 at the 8-week time point. mRNA levels were elevated in both the ipsi- and in the contralateral DRG (but more so in the ipsilateral DRG). mRNA levels for substance P or for the NGF receptor, TrkA, were not elevated (Figure 1). mRNA levels for the genes studied were not elevated in *Adamts5* KO mice.

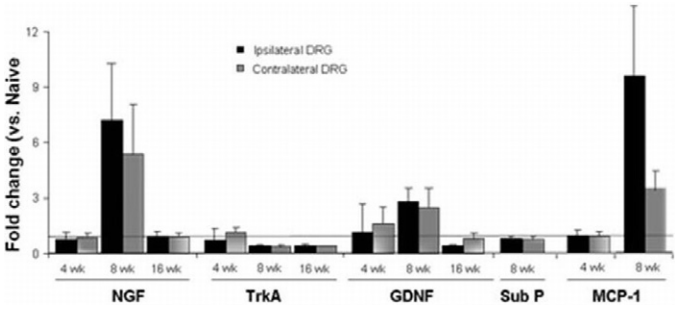

Figure 1

Conclusions: Time-course experiments in the murine DMM model of OA enable us to quantify pain at different stages of disease. Pain-related outcome measures in the DMM model can essentially be divided into an early stage (wk 2-8, allodynia only) and a late stage (wk 8-16, resolution of allodynia and appearance of reduced locomotion). Increased mRNA levels for neurotrophic factors and for MCP-1 in the innervating DRG were detectable 8 weeks after surgery. These observations suggest that the DMM model is suitable for studying mechanisms of chronic OA pain generation.

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RUNT-RELATED TRANSCRIPTION FACTOR 2 ALLELES AND KNEE CARTILAGE LESIONS ARE ASSOCIATED WITH INCREASES IN KNEE PAIN OVER 5 YEARS IN OLD ADULTS

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Purpose: Runt-related transcription factor 2 (RUNX-2) is the pivotal transcription factor for chondrocyte hypertrophy. This study aimed to determine if RUNX-2 and knee cartilage lesions were associated with change in knee pain over 5 years in older adults.
Methods: A total of 755 randomly selected subjects (mean 62 years, range 51-81, 50% female) were studied at baseline, 2.9 and 5 years later. Knee pain (on flat surface, going up/down stairs, at night, sitting/lying and standing upright) at baseline and 5 years was assessed using WOMAC. RUNX-2 alleles (BB, Bb and bb) were genotyped at baseline. Fat-suppressed MRI of the right knee was performed to determine knee cartilage volume and defects at baseline and 2.9 years.
Results: In multivariable analysis, RUNX-2 b allele (Bb 11.8% and bb 1.4%) was associated with a borderline increase in total knee pain score ($\geq$1) (OR 1.89, P=0.06), an increase in knee pain at night (OR 2.61, P=0.012) and an increase in sitting/lying knee pain (2.51, P=0.028) over 5 years. Increase in total knee pain score over 5 years was associated with female sex (OR 2.2, P=0.03), body mass index (OR 1.07 per kg/m^2, P=0.004), knee radiographic osteoarthritis (OR 2.03 per grade, P=0.006), medial tibiofemoral cartilage defects (OR 1.46 per grade, P=0.006), lateral tibiofemoral cartilage defects (OR 1.38 per grade, P=0.025), change in medial cartilage defects over 2.9 years (OR 1.68 per grade, P=0.004) and loss of medial tibial cartilage volume over 2.9 years (OR 1.07 per percent loss, P=0.03).
Conclusions: Multiple factors contribute to an increase in knee pain over 5 years. This is the first study to report that the RUNX-2 b allele predicts increased knee pain. Cartilage lesions are related to knee pain, independently of potential confounders.

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THE CATHEPSIN K INHIBITOR AZ12606133 REDUCES ARTICULAR CARTILAGE BREAKDOWN AND JOINT PAIN IN OSTEOARTHRITIC KNEES

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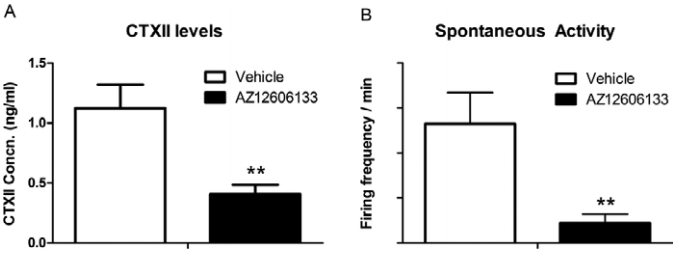
Purpose: The aim of the present study was to evaluate the chondroprotective and pain modulating effects of a novel cathepsin K inhibitor AZ12606133 in the Dunkin-Hartley guinea pig model of spontaneous osteoarthritis (OA).
Methods: Nineteen mature (9 month old) Dunkin Hartley guinea pigs were chronically implanted with an Alzet osmotic pump (2.5μl/hr; total volume = 2ml) which was set to deliver either the cathepsin K inhibitor AZ12606133 (0.5μg/kg/day, n=10) or vehicle (n=9). On day 28 of treatment, urine was collected over a 6 hour period by placing guinea pigs in metabolism cages. The urine was stored at -20[[Unsupported Character - ]]C until later analysed for evidence of cartilage degradation using the cross-linked C-telopeptides of type II (CTXII) collagen assay. Animals were then prepared for electrophysiological recording of knee joint primary afferent nerves. Recordings from mechanosensory nerves were made at rest (spontaneous activity) and in response to rotation of the knee either in the normal working range (40mNm) or the noxious range (60mNm).
Results: AZ12606133 caused a 63% reduction in urinary CTXII levels compared to vehicle treated guinea pigs (Figure A). Spontaneous afferent firing rate in vehicle-treated animals was 52.9±14 action potentials/min (n = 26



fibres) compared to only 8.8±4 action potentials/min (n = 18 fibres) in the AZ12606133 treated cohort (Figure B). Movement-evoked nerve firing in AZ12606133 treated animals was approximately 50% of the control group.
Conclusions: Chronic treatment of OA animals with AZ12606133 caused a reduction in articular cartilage destruction as well as attenuating joint nociception. It is possible, therefore, that chondroprotection can have beneficial effects on OA pain severity.

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MMP-13 INHIBITORS REDUCE NOCICEPTION IN A RAT MODEL OF OSTEOARTHRITIS

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Purpose: MMP-13 is a major collagenase in osteoarthritic cartilage, and specific inhibitors of it reduce structural deterioration in animal models of osteoarthritis. Broad-spectrum MMP inhibitors reduce pain behavior as well as structural damage in such models, apparently by independent mechanisms. The purpose of the present study was to determine whether MMP-13-specific inhibitors affect pain behavior in rats following anterior cruciate ligament transection (ACLT), and if so, to begin assessing possible mechanisms of action.
Methods: Three MMP-13 inhibitors were tested, with IC50's against the rat enzyme of 41 (compound A), 7 (compound B), and 4 nM (compound C). All three are at least a thousand-fold less potent against MMPs 1, 2, 3, 8, 9,12, and 14, and all three were negative in assays for inhibition of COX-1 and COX-2. Sprague-Dawley rats were subjected to ACLT in the right knee. The rats were then divided into five groups of 12, 10 of which were used for sensory testing. The groups were dosed orally, b.i.d., beginning the day of surgery, with: vehicle, compound A (60 mg/kg), compound B (30 mg/kg), compound C (30 mg/kg), or meloxicam (a commercially available cyclo-oxygenase inhibitor) (0.5 mg/kg). Laboratory personnel were blinded with respect to compound identity. Once each week following surgery, for 4 weeks, the rats were tested for "use-induced" pain: The length of time required for a tail-flick response to a high-intensity beam of light was determined before and 1, 3 and 6 minutes after repetitive flexion and extension of the right knee. A reduction in the time-to-tail-flick is assumed to reflect pain in the agitated joint. After the final set of these measurements, serum was taken to determine drug levels and the joints were taken for histological analysis.
In*in vitro* experiments, cleavage of pro-IL-1β was detected by western blot and IL-1 activity was determined using Jurkat cells transfected with the IL-1 receptor and a luciferase reporter.
Results: With vehicle-treated rats, joint agitation decreased the time-to-tail-flick at all test times. By the fourth week of the study, all three MMP-13 inhibitors completely eliminated this decrease, as did meloxicam. In the first week, only meloxicam was clearly effective. Compound C was fully effective by the second week, compound B by the third week, and compound A not until the fourth week. The compound that acted most quickly, compound C, was the most potent against MMP-13 and showed the highest serum concentration at the time of sampling. The histological analysis revealed little loss of collagen in this experiment. IL-1β is a known inducer of pain, and in *in vitro* experiments, MMP-13 was found to generate active IL-1β from the precursor.
Conclusions: Inhibiting MMP-13 reduced the pro-nociceptive effects of repetitive flexion and extension of the knee in the rat ACLT model of osteoarthritis. Other MMPs have been reported to induce pain by activating IL-1β, and we found that MMP-13, too, can generate an active form of this cytokine.

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PARTIAL JOINT IMMOBILISATION PROTECTS AGAINST OA AND REVEALS DISTINCT BIOMECHANICAL THRESHOLDS IN THE JOINT

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Purpose: The role of mechanical factors in OA is undisputed, but how these factors drive the processes that lead to joint disease is unknown. What has emerged in recent years is that OA is not simply a disease of cartilage attrition as a result of abnormal or repetitive wearing of the surfaces, but it requires activation of pathways that lead to expression of proteases which degrade the matrix. We used a surgical model of murine OA, induced by destabilisation of the medial meniscus (DMM) to examine the early expression of inflammatory genes in the joints of OA mice and to assess the influence of joint loading on gene expression and the development of OA.
Methods: DMM surgery was performed on 10 week old male C57Bl6 mice or FGF2 null mice by cutting the right menisco-tibial ligament. Some mice underwent sham surgery where the capsule of the joint was opened but the menicso-tibial ligament was left intact. The mice were either left for up to 12 weeks and joints sectioned for histological scoring, or had RNA extracted at early time points post surgery. Microarray analysis was performed and regulated genes were selected and validated quantitatively by RT-PCR using Taqman high density microfluidic cards. A subtotal reduction in weight bearing through the ipsilateral hind limb was induced by cutting the sciatic nerve at the time of DMM surgery. Complete joint immobilisation following DMM surgery was achieved by prolonged anaesthesia.
Results: Compared to sham operated mice, DMM surgery strongly induced a number of genes within 6h of surgery. Of these, the chemokine CCL2, TNF-stimulated gene 6 (TSG-6), IL-6, serum amyloid A (SAA) and arginase 1 were the most highly regulated. Metalloproteinases including ADAMTS4, ADAMTS5 and MMP3 were also regulated. Mice that had undergone sciatic neurectomy exhibited abnormal gait; some weight was born through the limb, but the leg was maintained in full extension and walking was achieved by flexion at the hip. When DMM surgery was performed at the same time as sciatic neurectomy the joints showed no evidence of OA even 12 weeks post surgery. Analysis of early gene expression in these animals revealed a striking abrogation of 70% of the measured genes. Complete joint immobilisation (by prolonged anaesthesia) following DMM surgery abrogated all gene responses in the joint. Those genes that were abrogated by complete immobilisation, but still induced following DMM surgery in neurectomised mice were shown to be highly FGF2-dependent in vivo.
Conclusions: These data show that there is an early inflammatory response in the joint to DMM surgery which is highly mechanosensitive. The selective abrogation of genes following partial and complete joint immobilisation identifies at least two different in vivo mechanical thresholds within the joint, one of which appears to be mediated by FGF2. We hypothesise that exceeding these thresholds determines whether protective or degradative pathways are activated and whether OA develops.

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EFFECTS OF EXERCISE PROGRAM ON PAIN, JOINT STIFFNESS AND PHYSICAL FUNCTION IN ELDERLY PATIENTS WITH KNEE OSTEOARTHRITIS. NURSING-BASED VERSUS HOME-BASED EXERCISE

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Purpose: Osteoarthritis (OA) is a leading cause of pain and disability in elderly people. The knee is the most frequently debilitating joint involved, compromising function and independence. Exercise can be beneficial in reducing physical and functional problems experienced by people with OA. Strength and resistance training are potentially relevant to knee OA because quadriceps weakness has been related to the development and progression of knee OA and is modifiable by training. Therefore, the aims of this study were to evaluate the effects of a progressive 8 weeks resistance-training program in subjects with knee OA with respect to pain, joint stiffness and physical function and to verify if differences exist between exercise done in group by nursing resident's subjects (nursing-based) or individually by subjects living in their own home (home-based).
Methods: Sixty seven subjects (25 men; 42 women) were divided into two groups: an exercise group (ExG) (n=34), mean (SD) age 75.2 (4.9) yr, body mass 72.1 (10.7) kg, height 160.9 (8.4) cm, body mass index (IMC) 27.4 (3.6) $kg \bullet m^{-2}$; and a control group (CG) (n=33), age 74.9 (4.9) yr, body mass 74.1 (4.9) kg, height 160.4 (9.3) cm, body mass index (IMC) 28.5 (4.5) $kg \bullet m^{-2}$. The ExG was divided afterwards in a nursing-based group (n=19; 80.8±6 yr) and a home-based group (n=15; 69.5±3.8 yr). Diagnosis for knee OA was done according to American College of Rheumatology clinical and radiological criteria's. Resistance-training program involved lower extremity exercises, especially for quadriceps strengthening. The participants progressed from completing 2 repetitions per session 2 times per week, to