than did MIA-treated animals, whereas MIA-treated animals displayed greater hindpaw allodynia than did MNX-treated animals.

Conclusions: We have compared two commonly induced models of OA in rats, within a single experiment using identical assessment tools. Although both models display features of OA, including chondropathy, osteopytosis, synovitis and pain behaviour, differences between the models may indicate that they each uniquely reflect different aspects of human OA. In particular, the MNX model is characterised by greater synovitis and osteophyte formation, and persistence of osteochondral channels, whereas the MIA model is associated with more pronounced distal allodynia. Appropriate selection of OA models depends on relevance to the research hypothesis, and to OA phenotype which it is intended to model.

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DEVELOPMENT AND VALIDATION OF MECHANICAL ALLODYNIA AS A PAIN READOUT IN A PRECLINICAL MODEL OF OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is an age-related joint disease characterized by progressive degeneration of articular cartilage and development of chronic pain. Although a number of experimental models of OA have been employed to investigate the underlying etiologies of cartilage degeneration, there have been relatively few reports of the characterization of the chronic pain associated with animal models of OA. Moreover, the application of these models in the assessment of the analgesic efficacy of putative therapeutics remains largely unexplored. The purpose of the present study was to develop and validate mechanical allodynia as a pain readout with benchmark analgesics in the well-described and widely-used destabilization of medial meniscus (DMM) model of OA.

Methods: Male sv129 mice were anesthetized and medial meniscotibial ligament which anchors the medial meniscus to the tibial plateau was transected to induce OA. To determine optimal time-point for pain measurement, decrease in paw withdrawal threshold (PWT) [mechanical allodynia (pain)] was measured using von Frey filaments applied to the plantar surface of the hind paw once every week after surgery for 5 weeks. To evaluate analgesics efficacy, on day 28 after surgery animals treated with either duloxetine; celecoxib or diclofenac were tested for PWT 60 minutes following oral administration of the drug while animals treated with gabapentin were tested for PWT 180 minutes following oral administration of the drug.

Results: We have demonstrated that mice with DMM surgery were allodynic as early as day 7 after surgery and exhibited a decrease in PWT lasting through day 35. It was also observed that PWT in the sham group was low indicating surgical pain during the early days following surgery but PWT in sham animals recovered by Day 28 to normal values. Gabapentin and duloxetine fully reversed established pain in a dose-related manner while celecoxib and diclofenac partially reversed established pain in a dose-related manner reaching a maximum inhibition of 62% and 64% respectively at 30 mg/kg.

Conclusions: OA pain is a complex process involving both peripheral and central sensitization. Development of analgesics drugs for OA has been difficult partly due to the lack of appropriate animal models to evaluate pain in OA. We have reproduced and employed the mouse model of OA for pain to evaluate the analgesic properties of benchmark analgesics. These results suggest that this model could provide a tool to understand molecular pathways involved in induction and maintenance of chronic osteoarthritic pain and would also be useful in developing and evaluating drugs for relieving OA pain.

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BLOCKADE OF BOTH IL-1A AND IL-1B BY A COMBINATION OF MONOCLONAL ANTIBODIES PREVENTS THE DEVELOPMENT AND REVERSES ESTABLISHED PAIN IN A PRECLINICAL MODEL OF OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is a disease of the whole joint with the signature pathologic feature being articular cartilage loss leading to joint destruction. The clinical symptoms of OA are pain and functional impairment that includes joint stiffness and dysfunction. Interleukin-1 (IL-1) plays a major role in the development and progression of osteoarthritis (OA) both in terms of disease (structure) and symptoms (pain). IL-1α and IL-1β are two distinct cytokines that bind to the same receptor and are expressed in synovial membranes, cartilage, and synovial fluid of patients with OA. The purpose of the studies were to investigate the effects of blockade of both IL-1α and IL-1β by a combination of anti-IL-1α and anti-IL-1β monoclonal antibodies (mAbs) on a) the development of pain upon chronic dosing and b) established pain upon acute dosing in the DMM model of OA

Methods: Male sv129 mice were anesthetized and then medial meniscotibial ligament which anchors the medial meniscus to the tibial plateau was transected to induce OA. Intraperitoneal (ip) treatments with IL-1 neutralizing antibodies (mouse anti-mouse IL-1α mAb, mouse anti-mouse IL-1β mAb alone, or in combination) was initiated the day of the surgery and continued twice a week for 28 days (chronic dosing study) or started on day 27 after surgery and pain evaluated on day 28, 24 hours post-antibody dosing (acute dosing study). Pain was measured by decrease in paw withdrawal threshold of the injured hind limb (mechanical allodynia). Knees were harvested and histopathologic alterations were scored and characterized.

Results: Upon chronic dosing in the DMM model of OA, mechanical allodynia (pain) in animals treated with either anti-IL-1α (6 mg/kg) or IL-1β mAbs (6 mg/kg) was similar to mechanical allodynia observed in the vehicle control group. However, combination therapy with both antibodies at 6 mg/kg each significantly reduced mechanical allodynia. In this study, blockade of IL-1α and IL-1β also prevented cartilage degradation while blocking either IL-1α or IL-1β had no effect. Upon acute dosing, combination therapy with both antibodies at 6 mg/kg each significantly reversed established mechanical allodynia observed 4 weeks post-surgery.

Conclusions: Our preclinical data demonstrate that combination of mouse anti-mouse-IL-1α and anti-mouse-IL-1β mAb had significant beneficial effects on histopathological parameters of mouse OA and prevented development of pain as well as reversed established pain associated with OA. We have recently reported on a novel dual-specific biologics approach, termed Dual-Variable Domain-Immunoglobulin[TM] (DVD-Ig[TM]) that can convert two pre-existing mAbs into a dual targeting agent by combining the variable domains of two mAbs via naturally occurring linkers. These preclinical proof of concept studies form the basis for further investigation of therapeutic IL-1α/β DVD-Ig[TM] molecules in human OA patients.

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ARTICULAR CARTILAGE DEGRADATION INDUCED BY EXTENSIVE TREADMILL EXERCISE IS GREATLY EXACERBATED BY ESTROGEN DEPLETION IN MICE.

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Purpose: Osteoarthritis (OA) is considered to be a multifactorial disease with factors such as chronic inflammation, aging, menopause, obesity, and joint instability. For example, more than 39% of the patients develop OA in the knee joint after anterior cruciate ligament reconstruction within 8 years. Too much mechanical stress by treadmill exercise induces severe articular cartilage degradation in rats. Estrogen depletion by ovariectomy (OVX) accelerates cartilage degradation in mice. These data clearly show that each factor is involved in the pathogenesis of OA, however, orchestrated effects of all these factors are still unclear. To analyze the crosstalk between estrogen signal and mechanotransduction in articular cartilage homeostasis, we examined articular cartilage damage after treadmill exercise in OVX mice. Here we report that degree of articular cartilage degradation induced by extensive treadmill exercise is greatly exacerbated by estrogen depletion.

Methods: Twenty-four Balb/c mice (8 wks) were randomly divided into 4 groups, OVX+CAGE, OVX+RUN, SHAM+CAGE and SHAM+RUN. Two weeks after ovariectomy or sham operation, RUN group was subjected to a forced running for 6 weeks (5 days a week) at 20m/min for 100 minutes by treadmill while CAGE group were left in cage ad libitum. Integrity of articular cartilage was assessed by Hematoxylin and Eosin staining and type II collagen immunostaining of sagittal sections. To assess articular

cartilage damage, three sections (apart from 150μm respectively) were stained by Safranin O and 400μm 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 Axio Vision Image Analysis system. To analyze the structural alteration of knee joint after treadmill exercise and OVX, right knee joints were fixed in 70% ethanol and subjected for μCT analyses. Animal care and experimental procedures were in accordance with the guidelines of the ethics committee of our university. Kruskal-Wallis test followed by Tukey-Kramer methods was used for statistical analysis.

Results: μCT analyses did not reveal the apparent alteration in bone structure and osteophyte formation between OVX and SHAM group regardless of treadmill exercise. However we observe significant loss in trabecular bone volume in the OVX group. Image analyses of the articular cartilage indicated that % area of Grade III was significantly higher in OVX+RUN group (72.1%) if compared to that of SHAM+CAGE (10.6%, p<0.05). Loss of proteoglycan in articular cartilage was also observed in both OVX+CAGE and SHAM+RUN groups (Grade III; 24.2% and 20.1% respectively) although the difference was not statistically significant. Immunohistochemical staining for type II collagen further support these data, since we observe marked decrease in type II collagen amount in OVX+RUN group. Hematoxylin and Eosin staining showed that OVX enhanced cellularity of synovial membrane after forced running suggesting that depletion of estrogen might enhance syovitis in the damaged joint.

Conclusions: In this study, we showed that the combination of OVX and forced running greatly accelerated articular cartilage denaturation in mice. In contrast, the effects of OVX or forced running respectively were not so apparent on articular cartilage maintenance. These data strongly support the evidence that OA is a multifactorial disease and our data strongly indicate the great contribution of hormonal regulation on articular cartilage homeostasis in adults. Crosstalk between hormonal regulation and mechanotransduction on articular cartilage homeostasis is still unclear, our data suggest that estrogen depletion enhances inflammatory response after articular cartilage damage. Since prevalence of OA greatly increased after menopause, our experimental system will be of great use for analyzing OA progression in aged women.

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INCREASED SENSITIVITY TO NERVE GROWTH FACTOR IN THE MONOSODIUM-IODOACETATE MODEL OF OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is often characterised by episodes of increased pain with associated synovitis. Nerve growth factor (NGF) levels are increased in the OA joint, and during inflammation. Pain flares may be due to either increased release, or to increased sensitivity to NGF. It was hypothesised that OA leads to a sustained and enhanced increase in NGF-induced pain behaviour. The Aim of this study was to find out whether pain behaviour induced by intra-articular injection of NGF is increased in the MIA model of OA and to explore possible mechanisms behind this enhanced response to NGF.

Methods: OA was induced in the left knee joint on day 0 in male Sprague Dawley rats (n=8 animals/group) weighing approximately 200g, by intra-articular injection of 1mg of MIA in 50μl saline. Saline injected animals were used as non-arthritic controls. Intra-articular injection of NGF (10μg/50μl) or saline control was given in the left knee joint when OA pathology had fully established (day 20). In order to explore whether inflammation during the development of OA may contribute to any increased sensitivity to NGF, indomethacin (2mg/kg, daily, orally) or saline control was given from before induction of OA (day -1) to day 18. To avoid confounding by analgesic actions of indomethacin, animals underwent a 2 day washout period before NGF injection. Joint tissues were harvested from parallel groups of animals at day 20, corresponding to the time of NGF injection in order to explore mediation of indomethacin effect through altered joint structure. Synovial inflammation was measured as the macrophage fractional area (% synovium occupied by ED1+ve cells), thickness of the synovial lining and joint swelling. Chondropathy, channels breaching the

osteochondral junction (OCJ) and osteophytes were scored as measures of structural damage. Pain behaviour was measured as hind-limb weight-bearing asymmetry. Data are reported as the mean [95% confidence interval].

Results: Pain behaviour was increased following induction of OA. Pain behaviour was reduced during the period of indomethacin treatment in OA animals to saline injected control levels by day 18 whereas vehicle-treated arthritic controls still showed an increase in pain behaviour. Enhanced and sustained pain-response was observed following intra-articular injection of NGF in OA animals (31.4 [24.6-38.2] g compared with that observed in non-arthritic controls (7.1 [4.3-9.8] g, p<0.001). Pre-treatment with indomethacin significantly inhibited the subsequent pain behavioural response to intra-articular NGF injection in OA knees (17.3 [12.1-22.5] g) compared to vehicle treated arthritic controls (31.4 [24.6-38.2] g, p<0.001). Synovial inflammation, chondropathy, channels breaching the OCJ and osteophyte scores at day 20 (before NGF injection) were not affected by indomethacin treatment.

Conclusions: NGF-induced pain behaviour is increased and sustained in the MIA model of OA. Pre-treatment with indomethacin reduced this enhanced response. This effect of indomethacin was not associated with any reduction in joint damage nor any significant sustained reduction in inflammation. Although synovitis may contribute to the development of this enhanced sensitivity to NGF, involvement of other factors such as possible effects of indomethacin on pain processing at the level of the spinal cord, deserve further investigation.

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INHIBITION OF TRANSFORMING GROWTH FACTOR ALPHA SIGNALING SLOWS PROGRESSION OF OSTEOARTHRITIS IN A DMM MODEL

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Purpose: No cures currently exist for the degenerative joint disease osteoarthritis (OA). Furthermore, the complex and multi-variable nature of OA continues to challenge the development of effective therapies. In an attempt to identify potential targets for disease modifying osteoarthritis drugs (DMOADs), our lab recently established a surgical model of OA to study gene expression changes in degenerating cartilage. Transforming growth factor alpha (TGFα) gene expression was upregulated in our model and further in vitro studies showed that TGFα suppressed chondrocyte expression of anabolic factors aggrecan and type II collagen and increased expression of the catabolic factor matrix metalloproteinase 13 (MMP13). We thus identified TGFα as a novel therapeutic OA target. The purpose of this project is to examine the role of TGFα in the development of OA in vivo. We hypothesize that inhibition of TGFα signaling will delay disease progression in surgical OA models.

Methods: Ten week old male Tgfa null mice and control littermates received either destabilization of medial meniscus (DMM) or sham surgery. At 7 and 14 weeks post-surgery knee joint histopathology was assessed using the OARSI scoring method and tissues were immuno-stained for disease markers such as MMP13 and type II collagen neo-epitopes. In addition, we performed DMM and sham surgeries on six month old mice to create a more severe surgical model and assessed OA histopathology after 7 weeks. Lastly, we observed the development of spontaneous OA in eighteen month old Tgfa and control mice in a variety of joints.

Results: DMM surgery produced mild and moderate OA in ten week old mice after 7 and 14 weeks. Tgfa null mice had lower OARSI scores and expressed less MMP13 and type II collagen neoepitopes than their control littermates. When DMM was performed on six month old animals, severe OA was observed. In older mice however, there was no protection in the Tgfa KO group compared to controls. Eighteen month old mice developed mild spontaneous OA, but again there was no protection in the Tgfa KO group when compared to controls.

Conclusions: TGFα signaling plays an important role in osteoarthritis progression in vivo in mild surgical models. However, in severe OA models and in spontaneous OA development, lack of TGFα alone does not appear to be sufficient to prevent or delay disease progression. TGFα should be investigated further as a potential target for DMOAD development, especially in the acute post-injury stages.