Results: Preliminary data show that the medial and lateral tibial plateaus had significant difference in subchondral bone parameters especially at 24 week of age. The medial plateaus showed an increase in BMD up to 30 weeks and increases in SbpTh and trabecular thickness (TbTh) up to week 24. These parameters were significantly higher in the medial compared to the lateral site (BMD, $P < 0.05$; SbpTh, $P < 0.001$; TbTh, $P < 0.05$). The lateral plateau, showed a significant increase in bone volume/tissue volume (BV/TV) ($P < 0.05$), trabecular bone number (TbN) ($P < 0.01$) and a significant decrease in trabecular bone pattern factor (TbPf) ($P < 0.001$) between 16 and 24 weeks. Of all the bone parameters, only TbN ($P < 0.05$) was significantly higher in the lateral plateau while trabecular bone separation (TbSp) was significantly lower ($P < 0.001$), compared to the medial plateau. Macroscopic scores did not show any significant changes over the study period. But, microscopic grading showed mild to moderate cartilage damage which was higher in the medial than the lateral plateaus. There were some association between the severity of cartilage degeneration and subchondral bone changes tibial plateau.
Conclusions: Our preliminary data showing a significant increase in subchondral bone plate thickness in the medial compared to the lateral plateau support the theory that subchondral bone plate thickness in the medial site precedes changes in the lateral site in early OA. The subchondral plate thickening may be viewed as an attempt to compensate over compromised structural trabecular parameters and hence preserve mechanical properties, while the thicker trabecular bone may strengthen and compensate for the lack of connectivity found in the medial as compared to the lateral plateau.

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THE DIFFERENTIATION OF CARTILAGE CHANGES AFTER ANTERIOR CRUCIATE LIGAMENT TRANSECTION AMONG RABBITS OF DISTINCT AGES

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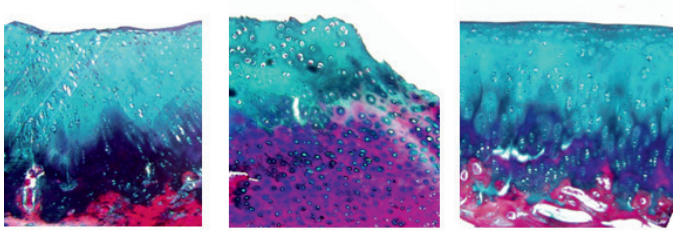
Purpose: In order to understand more about anterior cruciate ligament (ACL) injury and help dealing with ACL injury patients of different ages, we studied cartilage changes after ACL transection among rabbits of distinct ages.
Methods: In total, 30 rabbits comprising 3 age groups (A: 6 months, B: 12 months, C: 24 months, $n = 10$) were used for analysis. Each group was randomly divided into 5 groups ($n = 2$). Rabbits' right knees received ACL transaction, and the left knees were treated as control. Rabbits were sacrificed at the time of 5, 10, 15, 20 and 25 weeks after operation. We performed histological study (masson staining) for both knees of each rabbit and measured cartilage water ratio (WR) and gross injury score.
Results: We found that cartilage of the right knees degenerated more seriously than that of the left knees, and the cartilage of right knees degenerated fastest in group B while slowest in group C by macroscopic observation and masson staining. At 25 weeks after ACL transection, the cartilage degeneration was slightest in the rabbits of group C, more serious in group A, most serious in group B (Figure 1). There was a trend that the cartilage WR was increased and then decreased with the development of cartilage degeneration.
Conclusions: The significant different cartilage changes among distinct age groups suggest that the early intervention is necessary when dealing with young and middlescent ACL injury patients, but for old patients we may choose conservative treatment.



a b c

Fig. 1. Masson staining, 25 weeks after operation. (a) Group A; (b) Group B; (c) Group C.

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SIMULTANEOUS TARGETING OF IL-1A AND IL-1B BY A DUAL-VARIABLE-DOMAIN IMMUNOGLOBULIN (DVD-IG™) PREVENTS CARTILAGE DEGRADATION IN PRECLINICAL MODELS OF OSTEOARTHRITIS

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Purpose: Interleukin-1 (IL-1) plays a major role in the development and progression of osteoarthritis (OA). 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. IL-1 induces structural changes (cartilage degradation, bone sclerosis & synovial proliferation) by inducing proteases and proinflammatory cytokines from all tissues (cartilage, bone and synovium) of the joint. The purpose of the study is to demonstrate a) blockade of both IL-1α and IL-1β with a combination of anti-mouse IL-1α and IL-1β monocloncal antibodies (mAbs) is efficacious compared to treatment with single mAbs in mouse models of OA, and b) to demonstrate that anti-IL-1α/β dual variable domain-Ig (DVD-Ig) produces similar efficacy as the combination of anti-mouse IL-1α and IL-1β mAbs.
Methods: Interleukin-1 (IL-1) plays a major role in the development and progression of osteoarthritis (OA). 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. IL-1 induces structural changes (cartilage degradation, bone sclerosis & synovial proliferation) by inducing proteases and proinflammatory cytokines from all tissues (cartilage, bone and synovium) of the joint. The purpose of the study is to demonstrate a) blockade of both IL-1α and IL-1β with a combination of anti-mouse IL-1α and IL-1β monocloncal antibodies (mAbs) is efficacious compared to treatment with single mAbs in mouse models of OA, and b) to demonstrate that anti-IL-1α/β dual variable domain-Ig (DVD-Ig) produces similar efficacy as the combination of anti-mouse IL-1α and IL-1β mAbs.
Results: In both JIM and DMM model, cartilage degeneration in animals treated with either anti-IL-1α (6 mg/kg) or IL-1β mAbs (6 mg/kg) was similar to vehicle control knees. However, a combination therapy with both antibodies (6 mg/kg each significantly decreased cartilage degeneration scores. We have recently reported on a novel dual-specific biologics approach, termed DVD-Ig™ that can convert two pre-existing mAbs into a dual targeting agent by combining the variable domains of two mAbs via naturally occurring linkers. Anti-mIL-1α/β DVD-Ig (6 mg/kg) also significantly inhibited the progression of OA with comparable efficacy to the combination of the parental mAbs.
Conclusions: Our preclinical data demonstrate that combination of mouse anti-mouse-IL-1α and anti-mouse-IL-1β mAb as well as anti-mouse-IL-1α/β DVD-Ig™ molecules had significant beneficial effects on histopathological parameters of mouse OA. These preclinical proof of concept studies with anti-IL-1α/β DVD-Ig™ molecules form the basis for further investigation of therapeutic IL-1α/β DVD-Ig™ molecules in human OA patients.

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THE TYPE III COLLAGEN TURNOVER AND NITROSYLATION IN A RAT MODEL OF COLLAGEN INDUCED ARTHRITIS (CIA). APPLYING THREE NOVEL ELISA'S MEASURING TYPE III COLLAGEN NEOEPITOPES

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Purpose: The chronic inflammatory disease, rheumatoid arthritis, primarily affects the synovial joints. Thickening of the synovial membrane is the main consequences of the inflammation, but alterations are also detected in cartilage and bone. During inflammation, nitric oxide levels are elevated resulting in increased post-translational modification of extracellular proteins, such as nitrosylation of tyrosines. Thickening of the synovial membrane, which is caused by increased cell numbers, lead to elevated levels of proteolytic enzymes. This lead to increased degradation of the surrounding tissue, increasing the turnover of extracellular matrix, e.g. type III collagen. In this experiment we investigated whether nitrosylation of tyrosine of the synovial membrane is dependent of the turnover of the tissue (detected as type III collagen turnover).
Methods: Collagen induced arthritis (CIA) was induced in 10 Wistar rats by immunizations with 450 µl 2 mg/ml porcine type II collagen dissolved in 0.05M acetic acid and emulsified 1:1 in Incomplete Freunds Adjuvant

on day 0 and 7. 10 Wistar rats were used as control, with injection of 0.05M acetic acid. On day 16 all 10 CIA rats were diseased, determined by swollen paws. All the rats were scarified at day 26. Blood was taken at baseline, during the experiment and at termination and used for biomarkers measurements. Disease onset was defined as the day with significant difference between the paw score (swelling of paws) of SHAM and CIA. ELISAs were used to quantitatively measure turnover of type III collagen, CO3–610 to detect degradation and PIIINP to detect formation. A novel competitive ELISA (s-NysCo3) was used to detect nitrosylation of mature type III collagen.

Results: A 40% increase in degradation of type III collagen was detected in CIA rats after disease onset, but no significant increase in formation of type III collagen was detected. However, there was a tendency of increased formation of type III collagen in CIA rats after disease onset. These results led to a 40% increase of type III collagen turnover in the diseased CIA rats and this increase of turnover was already, though not significant, detected at day 7. The nitrosylation of mature type III collagen was detected with at novel competitive ELISA, showing a tendency of increased nitrosylation of type III collagen in CIA rats after disease onset. However, when comparing the amount of nitrosylation to the amount of degraded type III collagen, a decrease in nitrosylation of type III collagen was detected.

Conclusions: Collagen induced arthritis in rats increased type III collagen turnover after disease onset and the level of nitrosylation of tyrosines was elevated compared to SHAM. However, the level of nitrosylation in CIA rats compared to the turnover of type III collagen showed lower a nitrosylation rate. This could be a direct effect of the increased amount of degradation of type III collagen in the CIA rats. In conclusion, the level nitrosylation of type III collagen is depended of the turnover of type III collagen.

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INHIBITION OF TRANSFORMING GROWTH FACTOR ALPHA SIGNALING SLOWS PROGRESSION OF OSTEOARTHRITIS

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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: Adult male Tgfa null mice and control littermates received either meniscotibial transection (MTX) or sham surgery. At 7 and 14 weeks post-surgery knee joint histopathology was assessed using the OARSI scoring method and tissues were immunostained for disease markers such as MMP13 and type II collagen neoepitopes. Since some features of cartilage and bone development are known to be recapitulated during the disease process, skeletal development in Tgfa null mice was also examined.

Results: MTX surgery produced mild and moderate OA in 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. Tgfa null mice also showed transient growth retardation after birth. Histological analyses demonstrated a wider hypertrophic zone of the growth plate. TRAP staining showed a decrease in osteoclasts at the cartilage-bone interface, suggesting that removal of hypertrophic cartilage is delayed. These phenotypes are absent in adult mice.

Conclusions: TGFα signaling plays an important role in osteoarthritis progression in vivo and should be investigated further as a potential target for DMOAD development.

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CORRELATION OF PAIN AND STRUCTURAL DAMAGES IN RAT MIA MODEL

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Purpose: Intra-articular injection of monoiodoacetate (MIA) in the rodent joint can cause chondrocyte death and subsequent cartilage degeneration. It can also lead to subchondral bone degradation and gait alteration. Therefore it is considered a useful osteoarthritis model to evaluate pharmaceutical and biologic agents to inhibit cartilage and bone degeneration and alleviate pain. The severity of pain/gait alteration and structural damage depend on MIA concentration and injection frequency. However it is unknown if there is any correlation between pain and structural damage over time. This study investigates how the pain/gait change correlates with structural damages longitudinally at two MIA doses in rat. It was hypothesized that the gait and structural changes would exhibit a similar trend during the post-MIA injection period.

Methods: Two groups of Sprague Dawley rats (n = 12/group) were injected with 0.5 mg or 2 mg of MIA (40 μl) on day 0 on their right knees. Gait analysis, incapacitance testing, and knee diameter measurement were performed before MIA injection and on days 3, 7, 14, 21, 28, and 35 days post-injection. Animals were sacrificed on days 21 (n = 4) and 35 (n = 8) for microCT and histopathologic evaluation. For microCT, the right knee from each animal was scanned at a nominal resolution of 18 μm using Scanco μCT80. Bone volume of distal condyles was determined. For histopathology, the right knees were trimmed into two equal frontal halves, fixed, decalcified, embedded in paraffin and sectioned. Sctions were stained with toluidine blue and examined microscopically. Cartilage matrix damage, bone resorption, bone sclerosis and osteophyte were scored for each sample. All data were expressed as Mean±SD. Two-way ANOVA and post-hoc Tukey's HSD testing or non-parametric testing were performed with $P < 0.05$ considered significant.

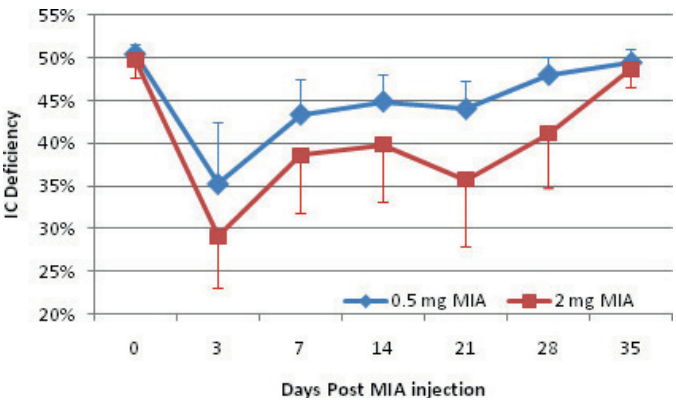

Fig. 1. Deficiency of right to total hindleg weight distribution by incapacitance test.

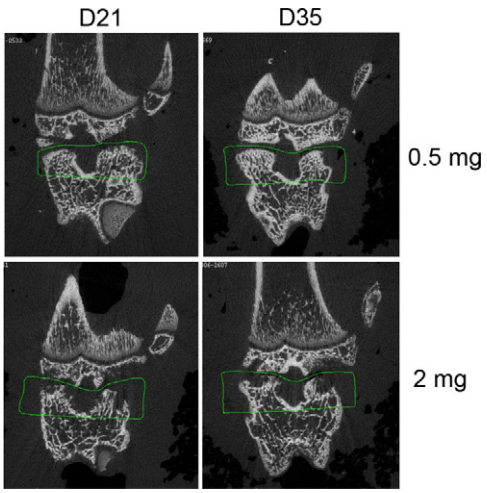

Fig. 2. Representative microCT images of distal femur.