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Purpose: The destabilisation of the medial meniscus (DMM) mouse osteoarthritis (OA) model is a commonly used model of post-traumatic OA. In this model, the knee joint is destabilised by transecting the medial-meniscotibial ligament which leads to increased loading on the medial tibial compartment, focal articular cartilage damage, subchondral bone accrual and osteophyte formation. In this study, we used the DMM-OA model to determine how tibial subchondral bone structure changes following acute joint injury and how these changes relate to onset and progression of articular cartilage damage.
Methods: 12-week old male C57BL/6 mice underwent DMM or sham surgery on the right knee; left knees served as contra-lateral controls. In vivo micro-CT (Skyscan) was performed prior to surgery, and at 4, 8, and 12 weeks post surgery. A novel approach for data analysis was developed to quantify bone of varying mineralization states. Separate groups of mice were collected for OARSI histologic assessment of bone structure, aggrecan loss and cartilage damage at 1, 4, 8 and 12 weeks post-surgery. Statistics: 2-way ANOVA, Bonferroni post-hoc tests.
Results: Pre-surgery bone volume/tissue volume (BV/TV) and tissue mineral density (TMD) were similar in all limbs. Consistent with increased loading, a focal increase in medial subchondral bone was observed in DMM-OA tibiae: BV/TV and TMD were increased compared to sham from 4 weeks post-surgery ($p<0.001$). There was no systemic effect of DMM-OA on bone structure: BV/TV in the lateral subchondral bone and tibial metaphyseal trabecular bone were similar in all limbs. However, medial subchondral BV/TV of DMM-OA tibiae and contra-lateral tibiae were similar, suggesting an influence of altered gait on the contralateral limb. Histologic analyses showed aggrecan loss and cartilage erosion in the medial compartment of DMM-OA tibiae from 4 weeks post-surgery. Interestingly, the medial subchondral bone in DMM-OA tibiae resembled osteonecrotic bone from 4 weeks post-surgery with numerous empty osteocyte lacunae present, indicating osteocyte death. Furthermore, the number of empty osteocyte lacunae at this site negatively correlated with the width of overlying aggrecan-positive (healthy) cartilage ($p=0.01$, Pearson's $r= -0.92$) suggesting a relationship between aggrecan loss and subchondral bone health.
Conclusions: In summary, focal accrual of medial subchondral bone occurs early in DMM-OA tibiae alongside cartilage damage. Similar to human OA, osteocyte cell death in subchondral bone is a feature of DMM-OA. Mechanical changes or biochemical signals induced by aggrecan loss in articular cartilage may be detrimental to the health of the underlying subchondral bone. Osteocyte cell death could also contribute to local changes in bone integrity, vascularization or cartilage. Finally altered bone structure in the contra-lateral tibiae of DMM-OA mice, suggests that increased subchondral bone per se does not affect overlying cartilage, and serves to highlight the need to include sham-operated mice when using this model.

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ELEVATED LEVELS OF BMP2 COMPENSATE FOR LOSS OF TGF-BETA ON PROTEOGLYCAN LEVEL IN ARTICULAR CARTILAGE DURING EXPERIMENTAL OSTEOARTHRITIS

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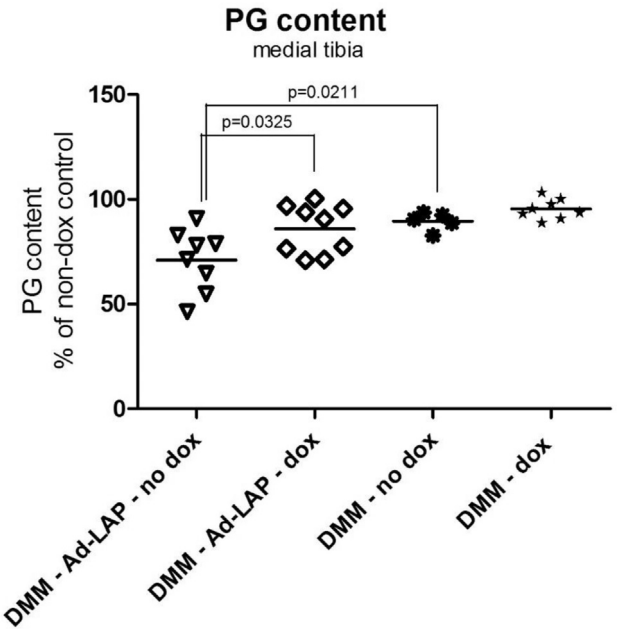
Purpose: We have demonstrated that in aging murine articular cartilage TGF-beta signaling via Smad2/3 is drastically reduced and that loss of Smad2/3-related TGF-beta signaling predisposed cartilage for OA development. In addition, we have previously shown that inhibition of TGF-beta reduces the proteoglycan content in articular cartilage. In contrast, during OA elevated levels of BMP2 are found in chondrocytes surrounding cartilage lesions. However, it is unclear what is the effect of this BMP2 presence on the articular cartilage. Therefore, we have investigated whether elevated BMP-2 expression can counteract the loss of TGF-beta signaling during OA.
Methods: We made a unique transgenic mouse which expresses human BMP2 under control of the Col2a1 promoter but only when exposed to doxycycline (Col2a1-rtTA-BMP2). This results in a chondrocyte-specific overexpression of human BMP2 which is inducible by doxycycline. Functionality of this transgenic mouse was tested by isolating mRNA from articular cartilage, spleen and liver 72 hours after exposure to doxycycline food or standard diet. With Q-PCR we analyzed the expression of human BMP2 mRNA. In Col2a1-trTA-BMP2 mice we induced OA by destabilization of the medial meniscus (DMM-model) while treating them with doxycycline in food versus standard diet. To study the effect of loss of TGF-beta activity during OA in these young mice, we additionally intra-articularly injected an adenovirus over-expressing the TGF-beta inhibitor LAP (Ad-LAP). Four weeks after induction of DMM knee joints were isolated for histology. OA was scored based on cartilage damage (adapted OARSI score, scale of 0-30) In addition, we measured proteoglycan (PG) content with digital image analysis in Safranin O stained articular cartilage of the medial tibia, which is most affected during DMM.
Results: Treatment of the Col2a1-rtTA-BMP2 transgenic mice with doxycycline clearly elevated the expression of hBMP2 mRNA in articular cartilage, but not in spleen and liver thereby confirming functionality of the transgenic animals. Doxycycline exposure in Col2a1-rtTA-BMP2 up to 8 weeks did not result in any detectible alterations in healthy articular cartilage. When OA was induced there was a clear increase in OA score (average of all DMM groups of 16.9 versus 2.5 in non-DMM groups), but this was not significantly affected by the presence of elevated chondrocyte-specific BMP2. TGF-beta inhibition with LAP did not affect the OA-score either . However, TGF-beta inhibition during DMM significantly reduced the proteoglycan content by 18% compared to DMM alone. BMP2 did not have an effect on the proteoglycan content during DMM (see figure). Nevertheless, the proteoglycan depletion that occurred by the inhibition of TGF-beta during DMM could significantly and nearly completely be counteracted by elevated chondrocyte-specific BMP2.



Conclusions: Our data show that in healthy articular cartilage and in cartilage affected by osteoarthritis in young animals elevated levels of BMP2 did not have any detectible effects. However, when TGF-beta signaling was lost, a phenomenon occurring in aged individuals, this resulted in decreased levels of PG content in articular cartilage during OA. In this setting, elevated levels of BMP2 could compensate this loss of PG. Therefore the elevated levels of BMP2 near OA lesions could be a reparative response of the articular cartilage. Especially with ageing, when TGF-beta signaling is drastically reduced this compensatory mechanism could be of great importance as an attempt to restore damaged articular cartilage. Overall our data show that with respect to proteoglycan content elevated levels of BMP2 can compensate for the loss of TGF-beta signaling, indicating that the presence of elevated BMP2 during OA could be a potential compensatory mechanism of the articular cartilage for the fact that TGF-beta signaling is lost. This potential attempt to repair the lost proteoglycans could be a mechanism of great importance especially in the elderly. Therefore our study provides novel insight into the mechanisms involved in OA.