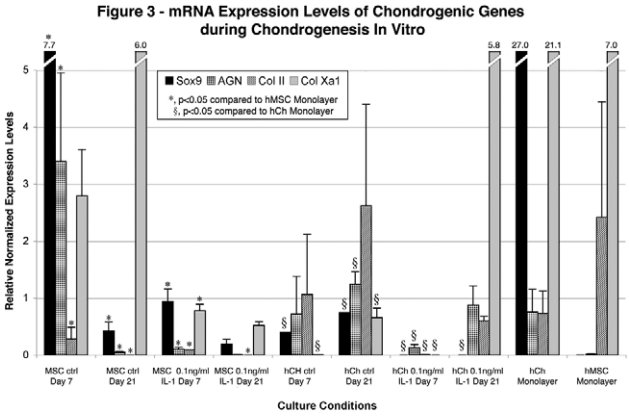

tive lineages, contributing to the inappropriate remodeling in OA. Taken together, our results suggest the Notch signaling pathway plays an important role in the pathogenesis of OA via potentially impairing the repair capacity of the diseased tissue.

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THE EFFECTS OF INTRA-ARTICULAR INJECTION OF P38 MAPK INHIBITOR ON MATRIX METALLOPROTEINASE IN CARTILAGE OF EXPERIMENTAL OSTEOARTHRITIS

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Purpose: The primary aim of this study was to investigate, using an experimental rat model of osteoarthritis(OA), the effect of a selective p38 mitogen activated protein kinase inhibitor,SB203580, on the development of structural changes.Additional aims were to assess the effects of the inhibitor on levels of matrixmetalloproteinase 3 (MMP-3) and MMP-13(collagenase 3) in OA cartilage and to explore the relation between the MMP-3,13 expression and the severity of OA.
Methods: OA was induced in 40 SD rats by anterior cruciate ligament transection (ACLT).After surgical, rats with OA were randomly divided into A_D groups: Rats of group A received 0.1 ml intra-articular injection of SB203580 at high concentration of 100um/L. Each treatment started immediately after surgery, once a week;those in group B were treated under the same condition using SB203580 with low concentration of 10um/L and those in group C received 0.1ml of intra-articular 0.9% Sodium Chloride injection,animals of group D were not injected as controls after ACLT. The animals were killed 8 weeks after surgery. Macroscopicand histologic studies were performed on the cartilage. The levels of MMP-3,13 in OA cartilage chondrocytes were evaluated by immunohistochemistry and western-blotting.
Results: All ACLT knees demonstrated osteoarthritic changes. Cartilage degradation in the control group was significantly more severe than that in the experimental group both on the macroscopic grading scale and on Mankin's grading scale($P < 0.05$). Immunohistochemical study showed that in the experimental group MMP-3,13 was predominantly expressed in the superficial and upper intermediate layers of cartilage, and the amount of MMP-3,13 in the experimental group was also lower than that in control group($P < 0.05$). In western-blotting the amount of MMP-3,13 was reduced by the treatment of the inhibitor. The protein levels of MMP-3 and MMP-13 in cartilage of inhibitor injection groups were significantly lower than those of Sodium Chloride group and untreated group. There was no significant difference in MMP-3 and MMP-13 expression between the different concentration inhibitor injection groups. No significant difference in MMP-3 and MMP-13 expression in cartilage was found between Sodium Chloride group and control group.
Conclusions: This study demonstrates that, in vivo,SB203580, a selective inhibitor of p38MAPK, can partially decrease the development of some of the structural changes in the early phases of experimental OA and significantly reduces the severity of cartilage degradation. This effect was associated with a reduction in the level of MMP-3,13 in OA cartilage, which probably explains the action of the drug and thus may be a potential drug for the treatment of OA.

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INVOLVEMENT OF TYROSINE PHOSPHORYLATION IN THE ACTIVATION PROCESS OF THE VOLUME SENSITIVE Cl^- CHANNEL IN RABBIT ARTICULAR CHONDROCYTES

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Purpose: Mechanical loading of articular cartilage influences the metabolism of extracellular proteoglycan and collagen matrix and thereby alters the osmotic environment surrounding chondrocytes. Previous studies have identified the presence of the stretch-activated cation channels, mechanosensitive K^+ channels and volume-sensitive Cl^- channels ($I_{Cl,vol}$) in chondrocytes, suggesting that these mechanosensitive ion channels contribute to cell volume regulation. Pathological swelling of chondrocytes observed in osteoaethritic (OA) cartilage may be ascribed at least partly to the impairment of channel function involved in the volume-regulatory process.The present study was designed to elucidate the mechanisms underlying activation of $I_{Cl,vol}$ in rabbit articular chondrocytes using whole-cell patch-clamp method.
Methods: Rabbit cartilages were collected from bilateral knee, hip and glenohumeral joints of male animals weighing 2.0 to 3.0 kg. The cartilage was dissected into slices and cultured in DMEM for 1-3 days, and chondrocytes were isolated by enzymatic digestion on the day of experiments. Whole-cell membrane current was recorded during exposure to isosmotic (300 mOsm), hyposmotic (210 mOsm) and hyperosmotic (350 mOsm) external solutions under conditions where Na^+, K^+ and Ca^{2+} currents were minimized. In some experiments, the stretch-activated cation current was also blocked by external application of 30 μM Gd^{3+}.
Results: Exposure of chondrocytes to the hyposmotic solution resulted in a cell swelling (27.0±2.7% increase in diameter, n = 28), which was accompanied by the activation of $I_{Cl,vol}$. External application of tyrosine kinase inhibitor genistein (30 μM) partially (42.0±14.3%, n = 7) and reversibly blocked $I_{Cl,vol}$ but its inactive analogue daidzein (30 μM) had no effect. On the other hand, intracellular application of tyrosine phosphatase inhibitor orthovanadate (250 and 500 μM) via a recording pipette gradually activated an outwardly rectifying current with a reversal potential (-20.3±0.58 mV, n = 13) close to the predicted Cl^- equilibrium potential (E_{Cl}- = -18.3 mV) even under isosmotic condition. This orthovanadate-evoked current was almost completely abolished by the stilbene-derivative Cl^- channel blocker DIDS (dihydro-4,4' diisothiocyanostilbene-2,2'-disulphonic acid, 500 μM) and was also largely reduced by cell shrinkage caused by exposure to hyperosmotic solution. These observations indicate that orthovanadate activates a Cl^- conductance which is sensitive to cell volume change. Pretreatment of chondrocytes with genistein significantly prevented the activation of Cl^- current by othovanadate, suggesting that the basal activity of tyrosine kinase is required for the orthovanadate-evoked activation of Cl^- current.

Conclusions: Taken together, our results strongly suggest that tyrosine phosphorylation is involved in the activation process of $I_{Cl,vol}$ in rabbit articular chondrocytes.

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LONG TERM NSAID TREATMENT DIRECTLY DECREASES COX-2 AND mPGES-1 PRODUCTION IN THE KNEE ARTICULAR CARTILAGE OF PATIENTS WITH OSTEOARTHRITIS

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Purpose: To simultaneously study the effect of a selective COX-2 inhibitor and that of a classic NSAID on the expression of pro-inflammatory genes in the articular cartilage of patients with severe knee osteoarthritis (OA) and in human osteoarthritic chondrocytes in culture.
Methods: A 3-month controlled, open clinical trial was carried out on 30 patients with severe knee OA scheduled for total knee replacement surgery. They were randomized into two groups: patients treated with celecoxib (CBX) (200 mg/24h) and patients treated with aceclofenac, a diclofenac proform, (DCF) (100 mg/12h). Patients with OA who did not want to be treated with NSAIDs served as the control group (CTR). After knee surgery, the articular cartilage was processed for molecular studies performed by western blot and real time PCR. In vitro studies were also conducted in chondrocytes isolated from OA joints. At second passage, these cells were used to examine the effects of CBX and DCF on proinflammatory gene expression in cells stimulated with 10 u/ml IL-1β.
Results: The gene expression of COX-2, mPGES-1 and iNOS was lower in the joint cartilage from patients treated with CBX and ACF. In the same way, at the protein level there was a reduction in COX-2 (CBX 0.3$\pm$0.1*; ACF 0.8$\pm$0.3; CTR 2.1$\pm$0.6; *$p<0.05$ vs. CTR), mPGES-1 (CBX 0.3$\pm$0.1*; ACF 0.3$\pm$0.1*; CTR 1.0$\pm$0.4; *$p<0.05$ vs. CTR) and iNOS (CBX 0.4$\pm$0.1*; ACF 0.3$\pm$0.1*; CTR 2.2$\pm$0.3; *$p<0.05$ vs. CTR) in the articular cartilage of OA patients. In cultured chondrocytes, we observed that both NSAIDs decreased the COX-2 and mPGES-1 synthesis as well as the PGE2 release induced by IL-1b. On the other hand, no effect was observed on NO or iNOS synthesis. With regard to the proinflammatory cytokines TNFa and IL-1b, which are involved in joint destruction, only CBX decreased the expression of both molecules (for TNFa: (CBX 1.0$\pm$0.1*,&; ACF 3.6$\pm$0.8; CTR 3.4$\pm$0.8; *$p<0.05$ vs. CTR; & $p<0.05$ vs. ACF; for IL-1b: CBX 1.9$\pm$0.6*,&; ACF 3.4$\pm$1.1; CTR 4.3$\pm$0.7; *$p<0.05$ vs. CTR; & $p<0.05$ vs. ACF) in the articular cartilage. However, both NSAIDs down-regulated IL-1b expression induced by cytokines in cultured OA chondrocytes.
Conclusions: Both NSAIDs diminished PGE2 release and unexpectedly, induced a decrease in COX-2 and mPGES-1 gene expression and protein accumulation in the cartilage from OA patients and in OA chondrocytes in culture. These data suggest that prolonged therapy with PGE2 blocking agents decreases PGE2 production not only by the direct inhibition of COX-2 activity, but also down-regulating COX-2 and mPGES-1 expression and synthesis in the articular cartilage. However, CBX and DCF seem to have a different anti-inflammatory profile in controlling cytokine gene expression in the cartilage.

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COLLAGEN IX IS INDISPENSABLE FOR PROPER GROWTH PLATE MORPHOLOGY AND ORGANISATION

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Purpose: The growth plate is known as a highly organized cartilage structure located between epiphyseal and metaphyseal bone at the distal ends in long bones, which enables longitudinal bone growth by endochondral ossification. This process is tightly controlled by the activity of the chondrocytes within the epiphyseal plate and regulated by a multitude of growth and transcription factors, the surrounding extracellular matrix, and nutrition. Collagen IX, as a component of the periphery of cartilage fibrils stabilizes and interconnects the fibrillar network with the extrafibrillar matrix. The current research was initiated to define the role of collagen IX for the cellular and fibrillar organization of the growth plate and its function in endochondral ossification.
Methods: Hind legs from wild type or *Col9a1*$^{-/-}$ mice at following embryonic (E) and postnatal (P) time points: E15.5, E17.5, P0.5 (newborn), P5.5, P15.5, and P50 were fixed in 4% paraformaldehyde (paraffin sections), or in 2.5% paraformaldehyde and 2% glutaraldehyde in 0.1M cacodylate buffer (semithin sections). Paraffin sections were stained immunohistochemically with antibodies against type X collagen, Sox9 and PCNA. Whole skeletons of newborn mice were stained with alcian blue and alizarin red. Results were evaluated according to histomorphometric standard procedures.
Results: The growth plate morphology of *Col9a1*$^{-/-}$ mice is markedly altered at all time points investigated, with changes being most prominent in late proliferative, pre-hypertrophic and hypertrophic and less obvious in resting and early proliferative zones. In the central region of the bones at early time points (E17.5 and P0.5) distinct areas with clearly reduced cell numbers and reduced glycosaminoglycan content are observed. The columnar arrangement is profoundly disturbed and aberrant proliferation of chondrocytes in horizontal direction was detected. In adult mice (P50) however, these alterations become attenuated and less prominent. Histomorphometric analyses reveal an irregular hypertrophic zone with a strongly decreased number of hypertrophic cells per area in the knockout, especially at later time points. Narrowing of this zone seems to be caused by a significantly reduced proliferation rate in the proliferating zone of the growth plate. The morphological changes are associated with shortening and broadening of all long bones in newborn *Col9a1*$^{-/-}$ mice. Moreover, the lack of type IX collagen alters the distribution pattern and diminishes number of Sox9 positive chondrocytes in central regions of the growth plate.
Conclusions: Collagen IX therefore plays a critical role for normal skeletal growth around birth and adolescent animals. However, we suggest that lack of collagen IX is compensated during growth since adult animals are similar in size to wild type mice.