centric 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 (ICl,vol) in chondrocytes, suggesting that these mechanosensitive ion channels contribute to cell volume regulation. Pathological swelling of chondrocytes observed in osteoarthritis (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 ICl,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 mM) hypnosomotic (210 mM) and hyperosmotic (350 mM) external solutions under conditions where Na+, K+ and Ca2+ currents were minimized. In some experiments, the stretch-activated cation current was also blocked by external application of 30 µM Gd3+.

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 ICl,vol. External application of tyrosine kinase inhibitor genistein (30 µM) partially (42.0±14.3%, n = 7) and reversibly blocked ICl,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 (ECV = -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′-disothiocyanostilbene-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 orthovanadate, 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
\( \text{IC} \), in rabbit articular chondrocytes.

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 pa-
tients 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 nM 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±0.1; ACF 0.8±0.3; CTR 2.1±0.6;
*p<0.05 vs. CTR), mPGES-1 (CBX 0.3±0.1; ACF 0.3±0.1; CTR 1.0±0.4; *p<0.05 vs. CTR) and iNOS (CBX 0.4±0.1; ACF 0.3±0.1; CTR 2.2±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-1β. On the other hand,
no effect was observed on NO or iNOS synthesis. With regard
to the proinflammatory cytokines TNFa and IL-1β, which are
involved in joint destruction, only CBX decreased the expression
of both molecules (for TNFa: (CBX 1.0±0.1*, ACF 3.6±0.8;
CTR 3.4±0.8; *p<0.05 vs. CTR; & p<0.05 vs. ACF; for IL-1β:
CBX 1.9±0.6*, &; ACF 3.4±1.1; CTR 4.3±0.7; *p<0.05 vs. CTR;
& p<0.05 vs. ACF) in the articular cartilage. However, both
NSAIDs down-regulated IL-1β expression induced by cytokines
in cultured OA chondrocytes.

Conclusions: Both NSAIDs diminished PGE2 release and un-
expectedly, 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 ac-
ivity, 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.

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 car-
tilage structure located between epiphyseal and metaphyseal
bone at the distal ends in long bones, which enables longitudi-
nal 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% paraformalde-
yde (paraffin sections), or in 2.5% paraformaldehyde and 2%
glutaraldehyde in 0.1M cacodylate buffer (semithin sections).
Paraffin sections were stained immunohistochemically with anti-
bodies against type X collagen, Sox9 and PCNA. Whole skele-
tons of newborn mice were stained with alcin 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 num-
bers and reduced glycosaminoglycan content are observed. The
columnar arrangement is profoundly disturbed and aberrant pro-
federation of chondrocytes in horizontal direction was detected.
In adult mice (P50) however, these alterations become attenu-
atated 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
and diminished 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.