ever. Se addition reduced this expression. In contrast, exposure to DON, NIV and T-2 toxin caused depressed TIMP-1 and TIMP-3 levels compared with the Control; this reduction was reversed with Se addition. Se addition also reversed the expression of α2 macroglobulin that was lowered by the three Toxins.

Conclusions: This study indicates that three toxins (DON, NIV and T-2 toxin) all depress type II collagen and aggregan expression in tissue engineered cartilage grafts cultured in vitro. The levels of MMP-1 and MMP-3 were increased but the levels of TIMP-1, TIMP-3 and α2 macroglobulin were all decreased in the presence of Toxins. Addition of Se reversed all of the expression effects produced by these Toxins. These in vitro results provide evidence for the potential biological mechanisms underlying cartilage degradation in the pathogenesis of KBD and how Selenium addition reverses these pathological effects.

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REGULATION OF MICROSOMAL PROSTAGLANDIN E2 SYNTHASE-1 AND 5-LIPOXYGENASE-ACTIVATING PROTEIN/5-LIPOXYGENASE BY 4-HYDROXYNONENAL IN OSTEOARTHRITIC CHONDROCYTES

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Purpose: Recently, we reported that the induction of cyclooxygenase-2 (COX-2) decreased dramatically after 24 hrs of incubation with 4-hydroxynonenal (4-HNE), a product of lipid peroxidation. This study aimed to investigate whether HNE is responsible for the shunt from COX-2 to 5-lipoxygenase-activating protein (FLAP)/5-lipoxygenase (5-LOX) in human osteoarthritic (OA) chondrocytes.

Methods: OA chondrocytes were treated with 10 μM of 4-HNE at different times of incubation (0 to 72 hrs). The protein level of mPGES-1 was evaluated by Western blot and that of prostaglandin (FLAP)/5-lipoxygenase (5-LOX) in human osteoarthritic (OA) chondrocytes.

Results: HNE induced the production of both PGE2 and LTβ4 by chondrocytes, but in opposite fashion. The level of PGE2 increased during the short period of stimulation (0-24 hrs), whereas that of LTβ4 increased after a long period of stimulation (48 and 72 hrs), where the level of PGE2 decreased. The Western blot data showed that protein expression of mPGES-1 increased gradually at 1000 μg/ml, reduced the level of MMP-1, PGE2 and IL-6. On the other hand, CS#1 was much less efficient in reducing the same catabolic markers and very surprisingly, in the absence of IL-1β, it increased the three catabolic factors tested, PGE2, IL-6, and MMP-1. As expected IL-1β inhibits the gene expression level of the collagen type II; only CS#3 was able to limit this inhibition. CS#1, in the presence or absence of IL-1β, further decreased collagen type II expression.

Conclusions: This study provides data on the effect of different CS on the cartilage metabolism. In this context, CS prescribed for alleviating OA symptoms should be taken with care as the origin, purity and/or production/purification of the CS compound could orientate the current OA disease process toward increased catabolic pathways.

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PATHOLOGY OF THE OUTERBRIDGE IV LESION; THERAPEUTIC IMPLICATIONS

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Purpose: The purpose of this study is to examine the gross and microscopic characteristics of the Outerbridge IV lesion that may serve as the foundation for cartilage repair.

Methods: Human osteochondral specimens having Outerbridge IV lesions were harvested following total knee surgery. They were subject to visual examination before and after Safranin O staining. Correlative histology was examined.

Results: The stained gross specimens showed cartilaginous aggregates on the surface as well as multiple small depressions. The microscopy showed cartilaginous aggregates on the surface breakdown of the cartilage in which pro-inflammatory cytokines and matrix metalloproteases (MMPs) are highly implicated. Previous studies have demonstrated that chondroitin sulfate (CS) exerts a protective effect on the cartilage. However, due to differences in CS in terms of origin, purity and the production/purification process, we compared the effects of three different types of CS on human OA cartilage.

Methods: Three types of CS were tested: namely CS#1 (porcine, purity: 90.4%), CS#2 (bovine, purity: 93.0%), and CS#3 (Bioibérica S.A.; bovine, purity: 99.9%). Treatment with each CS at 200 and 1000 μg/ml were performed in human OA cartilage explants in the presence/absence of IL-1β, and the protein modulations of factors, including PGE2, IL-6, and MMP-1, were investigated by specific ELISA. The CS effect on the expression of the pro-anabolic factor, collagen type II, was also investigated on OA chondrocytes using quantitative PCR.

Results: In the presence of IL-1β, CS#3 at 200 μg/ml, but not at 1000 μg/ml, reduced the level of MMP-1, PGE2 and IL-6. CS#2 followed the same trend as CS#3, however, at a much higher concentration, 1000 μg/ml. On the other hand, CS#1 was much less efficient in reducing the same catabolic markers and very surprisingly, in the absence of IL-1β, it increased the three catabolic factors tested, PGE2, IL-6, and MMP-1. As expected IL-1β inhibits the gene expression level of the collagen type II; only CS#3 was able to limit this inhibition. CS#1, in the presence or absence of IL-1β, further decreased collagen type II expression.

Conclusions: This study provides data on the effect of different CS on the cartilage metabolism. In this context, CS prescribed for alleviating OA symptoms should be taken with care as the origin, purity and/or production/purification of the CS compound could orientate the current OA disease process toward increased catabolic pathways.

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EFFECT OF THREE DIFFERENT CHONDROITIN SULFATES IN HUMAN OSTEOARTHRITIS CARTILAGE

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Purpose: During the osteoarthritis (OA) process, the altered metabolism of the chondrocytes is responsible for the extensive turnover of the articular cartilage. In this study, we investigated the effects of three different types of chondroitin sulfate on the metabolism of human OA chondrocytes.

Methods: OA chondrocytes were treated with 10 μM of 4-HNE at different times of incubation (0 to 72 hrs). The protein level of mPGES-1 was evaluated by Western blot and that of prostaglandin (FLAP)/5-lipoxygenase (5-LOX) in human osteoarthritic (OA) chondrocytes.

Results: HNE induced the production of both PGE2 and LTβ4 by chondrocytes, but in opposite fashion. The level of PGE2 increased during the short period of stimulation (0-24 hrs), whereas that of LTβ4 increased after a long period of stimulation (48 and 72 hrs), where the level of PGE2 decreased. The Western blot data showed that protein expression of mPGES-1 increased gradually at 1000 μg/ml, reduced the level of MMP-1, PGE2 and IL-6. On the other hand, CS#1 was much less efficient in reducing the same catabolic markers and very surprisingly, in the absence of IL-1β, it increased the three catabolic factors tested, PGE2, IL-6, and MMP-1. As expected IL-1β inhibits the gene expression level of the collagen type II; only CS#3 was able to limit this inhibition. CS#1, in the presence or absence of IL-1β, further decreased collagen type II expression.

Conclusions: This study provides data on the effect of different CS on the cartilage metabolism. In this context, CS prescribed for alleviating OA symptoms should be taken with care as the origin, purity and/or production/purification of the CS compound could orientate the current OA disease process toward increased catabolic pathways.
staining positive for mucopolysaccharides, type II collagen, and Lubricin. The depressions or pits were due to three conditions: aggregate erosion, vascular rupture, and dead bone fragmentation.

Conclusions: The cartilaginous aggregates have potential for proliferation contributing to cartilage repair. The multiple small pits could be the home for various cell therapies; i.e. synovial cells, stem cells, or therapeutics.

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SUSCEPTIBILITY OF CARTILAGE IN VIVO VERSUS CARTILAGE EXPLANTS IN VITRO

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Purpose: Joint bleeds lead to joint destruction. Knowledge about the mechanism of this blood-induced arthropathy has originated from both in vitro and in vivo studies. Our group has shown that in vitro exposure of cartilage to 50% v/v blood for 4 days leads to severe (-98%) and long-lasting (-78% after 16 days) inhibition in cartilage matrix synthesis. Also after an experimentally in vivo induced haemorrhage in the dog knee joint, direct harmful effects were observed, including inhibition of the cartilage matrix synthesis (-22%). But while in the in vitro experiments this inhibition was long lasting, in the in vivo experiments, effects were less outspoken and long-lasting. One of the differences between the in vitro and the in vivo situation is that in the in vivo situation, the cartilage is exposed to blood at the articular surface only, whereas in the in vitro explant culture system the cartilage is exposed to 5 additional cutting edges. Whether this difference in exposure of cartilage to blood can explain the difference between the in vitro and in vivo studies on blood-induced cartilage damage was subject of this study.

Methods: Human full thickness articular cartilage tissue was exposed to 50% v/v blood for 4 days either to all sides in an explant culture system, or in a culture system enabling isolated articular exposure (for this purpose a specific culture device was developed and validated). Subsequently the cartilage was cultured for an additional 12 days without blood to exclude the direct reversible effect. After these 16 days, cartilage proteoglycan synthesis rate and - content were determined.

Results: Exposure of cartilage to blood at all sides, both articular surface and cutting edges, led to a decrease in proteoglycan synthesis rate of -92% and a decrease in proteoglycan content of -19%. These effects were less outspoken when the cartilage was exposed to only the articular surface: -52% and -10% for proteoglycan synthesis rate and - content respectively.

Conclusions: In vitro exposure of cartilage to blood at the articular surface alone leads to less severe effects on the proteoglycan synthesis rate and - content than when cartilage explants are exposed at all sides. This is probably part of the explanation why blood-induced cartilage damage after an experimentally induced haemarthros in vivo is less severe compared to the in vitro effects of blood on cartilage. Irrespectively, blood has devastating effects on articular cartilage, and in this respect it is important to prevent (traumatic) joint haemorrhages and if they occur, to treat them properly. Additionally this study demonstrates that results of cartilage tissue explant cultures, exposed at all sides to culture medium and additions should be interpreted with caution.

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ACTIVATION OF VOLUME-SENSITIVE CHLORIDE CURRENT BY DOXORUBICIN IN ISOLATED RABBIT ARTICULAR CHONDROCYTES

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Purpose: Chondrocyte apoptosis contributes to the disruption of cartilage integrity in osteoarthritis (OA). Recently, it has been suggested that activation of volume-sensitive Cl- current (ICl,vol) mediates cell shrinkage triggering apoptosis (apoptotic volume decrease: AVD) in several cell types. The present study was designed to investigate the effects of a potent apoptosis-inducer, doxorubicin, on ICl,vol in rabbit articular chondrocytes using whole-cell patch-clamp technique.

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. On the day of experiments, chondrocytes were isolated by enzymatic digestion. Whole-cell membrane current was recorded under conditions where Na+, K+ and Ca2+ currents were minimized. Osmolality of bath solution was adjusted with mannitol. Real-time change in cell size was monitored using a CCD digital camera and the cross-sectional area of cell image was measured.

Results: Exposure of isolated chondrocytes to doxorubicin (1 μM) resulted in a gradual loss of cell size (approximately 7% decrease in the cross-sectional area over 30 min), which was significantly attenuated in the presence of a specific ICl,vol blocker 4-(2-butyl-6,7-dichloro-2-cyclopentylinden-1-on-5-ylo)xybutyric acid (DCPDB, 10 μM). On the other hand, whole-cell patch-clamp recording revealed an obvious increase in the membrane Cl- conductance by doxorubicin without any appreciable change in cell size. The doxorubicin-evoked Cl- current exhibited many properties characteristic of ICl,vol phenotype, including outward rectification, prominent inactivation at large positive potential (> +50 mV), inhibition by hyperosmotic cell shrinkage, and sensitivity to DCPDB.

Conclusions: The present results suggest that doxorubicin enhances the Cl- efflux via activation of volume-sensitive Cl- channel in rabbit articular chondrocytes, which may be involved in doxorubicin-induced AVD.