but was shifted temporally. qPCR results confirmed that the oscillation was also detectable at the RNA level.
Conclusions: Following dissection and culture, HtrA1 protein and RNA levels oscillate in articular cartilage. SIL also induces HtrA1 oscillations, but the oscillation is delayed in onset. We are currently investigating the mechanisms behind this response. Some evidence exists that cartilage dissection induces expression of cell cycle proteins, and we propose that HtrA1 may be involved in the control of the cell cycle in response to damage.

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STUDY OF THE O-LINKED-N-ACETYL-GLUCOSAMINYLATION OF PROTEINS INDUCED BY HIGH DOSES OF GLUCOSAMINE AND ITS CORRELATION WITH OSTEOARTHRITIS PROGRESSION

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Purpose: The addition of O-linked N-acetylglucosamine (O- GlcNAc) on serine/threonine residues on cytoplasmic and nuclear proteins is a post-translational modification that has been implicated in the regulation of a variety of signal transduction pathways. O-GlcNAcylation has been also shown to be deregulated in degenerative and age related diseases, such as Alzheimer, cancer and diabetes. In fact, we have previously demonstrated that human OA is characterized by the accumulation of O-GlcNAc modified proteins in the cartilage through the activation of the hexosamine biosynthesis pathway (HBP). Glucosamine sulfate (GS) is a natural glycosaminoglycan commonly used for the treatment of OA. Although its mechanism of action remains to be elucidated, it is known that glucosamine at high doses act as an inducer of HBP flux. The aims of this study were: (1) to determine whether an inductor of HBP flux could modify the amount of O-GlcNac modified proteins or the expression of the key enzymes responsible for GlcNAcylation: O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA); (2) to assess if this HBP manipulation could have any effect on the progression of cartilage damage in an experimental model of OA in rabbits.
Methods: We employed an experimental model of OA in rabbits induced by anterior cruciate ligament transection and partial medial menisectomy. OA was induced in 30 New Zealand rabbits that were sacrificed 12 weeks after surgery. 15 of those rabbits orally received GS (1500 mg/day) from 2 weeks before surgery until the end of the study. 8 additional rabbits were employed as healthy controls. At sacrifice, the hyaline cartilage from the medial tibia was extracted to carry out further protein expression studies. Some fragments were immediately frozen while others were digested in order to isolate chondrocytes. Furthermore, femurs were fixed and embedded in paraffin for histological analysis. Cyclooxigenase-2 (COX-2), OGT, OGA and the amount of O-GlcNAc modified proteins were assessed by western blot analysis employing specific antibodies. Cartilage lesions were quantified employing the Mankin score in sections stained with hematoxylin-eosin and alcian-blue PAS.
Results: We observed a significant increase in the amount of O-GlcNAcylated proteins in the cartilage of OA rabbits in comparison to healthy ones, a result that was in line with our data in human OA. In OA rabbits, GS treatment was able to significantly reduce the amount of O-GlcNAc modified proteins after 14 weeks of treatment. Regarding OGT protein expression, OA rabbits showed an increased presence of the short and long isoforms of this enzyme in the cartilage and in isolated chondrocytes, in comparison to healthy rabbits (Healthy: 0.6±0.1; OA: 1.7±0.2; $p<0.05$). However, GS treatment was unable to modify the presence of this enzyme in the cartilage of the rabbits (OA+GS: 0.4±0.1). Furthermore, OA induced a decrease in the presence of OGA in the cartilage that was not modified by GS administration either (Healthy: 10.8±1.6; OA: 1.4±0.6*; OA+GS: 1.1±0.3; * $p<0.05$ vs. H). GS treatment ameliorated cartilage damage induced by OA surgery and measured by the Mankin score (OA:9.1±1.3; OA+GS:5.5±0.6; $p<0.05$). A decrease was observed in both structural and cellular alterations induced by OA, as well as in the proteoglycan staining. GS also induced a significant inhibition in the protein expression of the proinflammatory mediator COX-2 in the cartilage of the rabbits (OA: 2.3±0.3; OA+GS: 0.4±0.1; $p<0.05$).
Conclusions: Our results demonstrate that a diminution in OA cartilage damage paralleled to a diminution in O-GlcNAc protein glycosylation, suggesting that O-GlcNAc glycosilation could have a phisio-pathological role in OA. However, GS was unable to modify the presence of the enzymes that regulate O- GlcNAcylation, wich points out to additional mechanisms of control of O-GlcNAcylation by GS.

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ANALYSES OF CARTILAGE SUPERFICIAL LAYER USING IMMUNOHISTOCHEMICAL STAINING

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Purpose: In recent years, many approaches to cartilage regeneration have been studied, but only chondrocyte and extracellular matrix(ECM), such as type 2 collagen and aggrecan, are focused as indicators of tissue regeneration. Although it is considered cartilage superficial layer (lamina splendens) is likely to play a key role in the function of articular cartilage, it is hardly evaluated, since little is known about the components of lamina splendens. Some studies suggested, in lamina splendens, collagen fibrils run parallel to the surface of articulation, and it is composed of type 1 and type 3 collagen instead of lack of type 2 collagen, which is the major type of collagen in articular cartilage. The purpose of this study is to analyze more detailed structure of lamina splendens and histological distribution of type 1 and type 3 collagen.
Methods: 1) Samples of pig normal articular cartilage were obtained from knee joint (tibia). Subchondral bone was cut off and non-decalcification paraffine specimens were made. After sectioning, they were treated with anti-type 1 collagen antibodies and anti-type 3 collagen antibodies. 2) Samples of human and pig normal articular cartilage were obtained from femoral head or tibia, and sliced one millimeter thick. Specimens were fixed in 2%GA-4%PFA solution and then postfixed in 1% osmic acid, embedded in epoxy resin, and observed under the transmission electron microscope (TEM). Other specimens were treated with anti-type 1 and anti-type 3 collagen antibodies after fixation in 0.05%GA-4%PFA solution, and embedded in epoxy resin(pre-embedding method). Specimens are observed using a TEM.
Results: 1) The superficial layer, about 10 um, was stained with both anti-type 1 and anti-type 3 collagen antibodies. 2) By electron microscopy, two layers were observed at the surface. One was high electron density layer, and another was low electron density layer, which contains microfibrils. Both of anti-type 1 and anti-type 3 collagen staining were observed in these two layers, but their size and shape were not uniform. Microfibrils in low electron density layer were not stained.
Conclusions: Although type 1 and type 3 collagen are contained in cartilage superficial layer, it is necessary to examine other molecules to determine the component of superficial layer.

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INHIBITION OF ASPORIN SIGNALING IS CRITICAL IN THE PREVENTION OF CARTILAGE DAMAGE BY PHYSIOTHERAPIES

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Purpose. Physiotherapies are the most frequently recommended non-invasive treatment options recommended for arthritic diseases. Here, we examined the signaling pathways responsible for the beneficial effects of exercise on various stages of monoiodoacetate-induced arthritis (MIA). We demonstrate that both success and failure of the physiotherapy is mediated via discrete signaling pathways involving Asporin, a susceptibility gene in osteoarthritis (OA). Asporin-mediated regulation of matrix synthesis in turn may regulate Transforming Growth Factor-β (TGF-β) networks essential for cartilage matrix synthesis.
Methods. Treadmill walking (TW), used as a means of exposing MIA afflicted knees to physiotherapy, was initiated in rats at different stages of MIA induced cartilage damage and compared to unexercised MIA afflicted knees. TW started at 1 day post-MIA induction, or after cartilage damage had progressed to Grade I or Grade II. The cartilage specimens were analyzed by macroscopic, microscopic, μCT imaging, transcriptome-wide gene expression analysis and activation/suppression of signaling