plateau) were collected, consisting in 1-mm-thick slices. Slices were cut tangentially to the articular surface, within the superficial and deep layers of the articular cartilage, respectively. Biochemical composition was assessed using Fourier-transform infra-red (FTIR), and morphological aspects were determined using scanning electron microscopy (SEM). All results are expressed as mean (SD).
Results: Age and body mass index at the time of intervention were 74.4 (8.3) years, and 28.8 (6.2) kg/m^2, respectively. Preoperative X-rays found uni-, bi- or tricompartmental knee OA in 2, 8 and 4 cases, respectively. In all cases, the compartment mainly affected was medial femoro-tibial, and Kellgren and Lawrence score was 3.8 (0.4). Joint calcifications were detected in only 2 cases on preoperative X-rays, but in 11 cases under macroscopic examination. FTIR analysis showed CC in all 14 specimens. CCs were absent from the control sample. The overall mineral content represented 9.1 (12.6)%. CCs were identified as BCPs only in 6 patients, and as both BCPs and CCPs in 8. Remarkably, CCs were widespread in all knee joint compartments and involved at least 3 areas out of 8 in all specimens. In addition, CCs distribution was similar between superficial and deep layers, and between medial and femoral compartments (11.1 [14.7]% vs 7.3 [10.2]%, and 8.1 [11.2]% vs 9.5 [13.1]%, p=NS, respectively). Finally, SEM identified 2 different morphological aspects: 1/spherical structures, typical of biological apatite, resulting from an agglomeration of nm-scale cristallites surrounded by proteins, and localized in structures suggestive of chondrons, in the BCPs-containing samples; 2/ acicular or cubic structures of different sizes, in the CPPs-containing samples.
Conclusions: CCs are constantly found in human OA articular cartilage at the time of knee joint replacement. Cartilage calcifications occur in all knee joint compartments, even in less weight-bearing ones, suggesting that cartilage mineralization process may reflect a generalized disease of the articular chondrocyte. In addition, their morphological aspects are specific to the crystal type.

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INTRACELLULAR CALCIUM OSCILLATIONS IN ARTICULAR CHONDROCYTES INDUCED BY BASIC CALCIUM PHOSPHATE CRYSTALS LEAD TO CARTILAGE DEGRADATION

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Purpose: Basic calcium phosphate crystals (BCP), including octacalcium phosphate (OCP), carbapatite (CA) and hydroxyapatite (HA), are associated with destructive forms of osteoarthritis. However, mechanisms of BCP-induced cartilage breakdown are poorly understood. We aimed to assess whether BCP induced intracellular calcium (iCa^{2+}) variations and the role of iCa^{2+} in BCP-induced glycosaminoglycan (GAG) release.
Method: Bovine articular chondrocytes (BAC) and cartilage explants (BCE) were stimulated by pyrogen-free OCP, CA, HA, or monosodium urate (MSU) crystals. iCa^{2+} levels in BAC were determined with the photoactive dye Fura-2, and iCa^{2+} oscillations by confocal microscopy. GAG release was measured in the supernatants of BCE cultures by DMMB assay. Mechanisms of BCP-induced iCa^{2+} oscillations were assessed using a pharmacological approach.
Results: All BCP, as opposed to MSU crystals, induced iCa^{2+} raise compared to baseline level (OCP: 2.4±0.9-fold control value, p<0.0001; CA: 2.4±1.8, p=0.003; HA: 1.4±0.4, p=0.005; and MSU: 1.5±0.6, p=0.160). OCP also induced iCa^{2+} oscillations within minutes (min). Ratio of BAC displaying iCa^{2+} oscillations increased with time to reach a maximum after 20 min of OCP stimulation (49.0±24.3%, p<0.005), whereas no iCa^{2+} oscillations were observed in control BAC stimulated only by microscopy laser beam or fluid flow. OCP-induced iCa^{2+} increase and iCa^{2+} oscillations involved extracellular Ca^{2+} (eCa^{2+}) influx and iCa^{2+} storage and exflux. Indeed, OCP-induced iCa^{2+} oscillations were abolished when experiments were performed in Ca^{2+}-free medium suggesting the role of eCa^{2+} influx. eCa^{2+} influx involved both voltage- and non-voltage-dependent Ca^{2+} channels, since iCa^{2+} levels decreased when BAC were pre-treated with either nickel (Ni) 5mM or verapamil 1 μM ((OCP+Ni)/OCP = 0.5±0.2, p=0.01; and (OCP+verapamil)/OCP = 0.6±0.2, p=0.01). Similarly, ratio of BAC displaying iCa^{2+} oscillations decreased rapidly when Ni was added, and was abolished by thapsigargin, an inhibitor of iCa^{2+} storage in endoplasmic reticulum. Finally, BAPTA 1 μM, an iCa^{2+} chelator, significantly decreased BCE GAG release induced by OCPs (1.1±0.4-fold the control value vs. 1.7±0.1, p=0.01, with or without BAPTA, respectively).
Conclusions: These results suggest that iCa^{2+} signaling is a major pathway involved in BCP effects on articular chondrocytes. They highlight a new pathophysiological mechanism and give rise to a new therapeutic target in diseases related to BCP deposition.

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IDENTIFICATION OF GENES REGULATED BY IL-1 USING INTEGRATIVE microRNA AND mRNA GENOMIC ANALYSIS IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: MicroRNA (miRNA)s are short endogenous oligonucleotides with a profound role in the regulation of post-transcriptional gene expression. miRNAs regulate their targets both by translational inhibition and acceleration of mRNA degradation. In this study, we attempted to identify differentially expressed miRNAs in human OA chondrocytes in response to IL-1. In addition, simultaneous profiling of miRNA and mRNA expression was performed to get an integrated analysis of miRNA and mRNA expression.
Methods: Articular cartilages were obtained from knee osteoarthritis patients and were cultured in monolayers. Chondrocytes were stimulated with interleukin (IL)-1 for 4 hours and RNA was isolated. One μg of total RNA was polyadenylated and converted to cDNA and miRNA microarray was performed. Seven hundred thirty five oligos were used, corresponding to 470 well-annotated human miRNA sequences and 265 potential miRNAs that were identified recently. mRNA microarray was performed simultaneously using the RNA samples that were used for miRNA array. Both sequence and expression information was used to identify regulatory relationship between miRNA and mRNA pairs.
Results: Expression profiling of 446 human miRNA extracted from IL-1 treated chondrocytes (*n*=6) identified 26 miRNA which showed significantly differential expression between control and treated cells. We also identified 7190 mRNAs differentially regulated by IL-1 treatment. Among the 26 miRNAs differentially regulated, 14 miRNAs had target searched by MiRANDA scheme. By combining target search and miRNA-mRNA pairing, we could identify 1224 miRNA-mRNA target pairs. The miRNA array data were corroborated by real time PCR analysis and in situ hybridization using samples from different donors.
Conclusions: MiRNAs regulated by IL-1 were identified in human articular chondrocytes with mRNAs they regulate. It is suggested that miRNA plays a role in the regulation of cartilage degradation in osteoarthritis.

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OSTEOARTHRITIS LEADS TO INCREASED LEVELS OF PROTEIN O-LINKED N-ACETYLGLUCOSAMINE IN THE CARTILAGE

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Purpose: The modification of serine/threonine residues on cytoplasmic and nuclear proteins by O-linked N-acetylglucosamine (O-GlcNAc) is a post-traslational modification that has been implicated in the regulation of a variety of signal transduction pathways. Despite the important role that has been described for this system in degenerative and age-related diseases, such as Alzheimer's and diabetes, the role of O-GlcNAcylation in osteoarthritis (OA) has not been considered previously.
The goal of this study was to determine the level of proteins modified by O-GlcNac in OA cartilage and chondrocytes and to analyze if there were alterations in the presence of enzymes critical in regulating O-GlcNAc levels, namely, O-GlcNAc transferase (OGT) and O-GlcNAcase.
Methods: Human cartilage was extracted from patients with knee OA during the joint replacement surgery (n=8), while healthy cartilage was obtained from the knee of age and sex-matched donors (n=5). We also employed an experimental model of OA in rabbits, induced by anterior cruciate ligament transection and partial medial menisectomy. OA was induced in 15 New Zeland rabbits, that were sacrifized 3, 8 or 12 weeks after surgery. 15 additional rabbits were employed as healthy controls. At sacrifice, the hyaline cartilage from the medial tibia was extracted and and immediately frozen. At the same time, all the femurs were fixed, decalcified and embedded in paraffin. Cartilage levels of O-GlcNAcylation,