Conclusions: In this study we tested our hypothesis that PRP has anti-inflammatory properties and influences gene expression of extracellular matrix forming and degrading proteins in an OA mimicking environment. An inflammatory environment was created through addition of IL-1 beta, one of the key players in OA pathogenesis, to chondrocytes in culture. Exposure of chondrocytes to the inflammatory cytokine IL-1 beta resulted in marked changes in the expression of genes involved in matrix formation and degradation as well as inflammation, many of which were reduced by the addition of PRP. The inhibition of NFkB activation was found to be a possible mechanism through which PRP exerted these effects. Revealing the relevant mechanisms and processes could improve application of PRP in a clinical setting. We consider these encouraging results for the further study of PRP as a conservative, autologous and cost-effective treatment for OA.

261 THE UNFOLDING PROTEIN RESPONSE (UPR) IS NORMALLY ACTIVATED BY BIOMECHANICAL STRESS IN CHONDROCYTES, BUT DYSREGULATION OF UPR ACTIVATION IS LINKED TO AGING AND OSTEOARTHRITIS PROGRESSION

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Purpose: OA is associated with aging and biomechanical stress. To identify chondrocyte abnormalities that provide a basis upon which OA is accelerated we studied the unfolded protein response (UPR), a fundamental means by which cells normally resolve stress. The UPR restores equilibrium to the stressed ER via a reprogrammed proteome rich in chaperones and protein folding catalysts. Three UPR signaling cascades are triggered by dissociation of distinct ER membrane proteins from the chaperone GRP78, which normally dampens the UPR, limits apoptosis, and promotes autophagy. Generation of the UPR-specific transcription factor XBP1s (spliced XBP1) is UPR-specific, and each UPR cascade promotes terminal expression of CHOP. Unsuccessful resolution of the UPR promotes oxidative stress, inflammation, and apoptosis (partly via XBP1s and CHOP). We tested if mechanical stress activates the UPR in chondrocytes, and probed for UPR impairment in aging and OA.

Methods: Normal bovine cartilage chondrocytes were placed into 3D alginate matrix molds and subjected to cyclic compression (maximum 22% height, 12% amplitude, 0.5 Hz) in a unique biomechanical reactor. Sections of human knee cartilage from OA and normal donors were analyzed in covered vs. uncovered tibial, and medial femoral condyle weight bearing vs. non-weight bearing zones. Chondrocytes were isolated from human knee OA (grades 2–4) and normal cartilages (grades 1) and compared for UPR mediator expression by microarray. Last, aging mouse OA 3T3L1/Skelet-6 weeks to 24 months) were analyzed for CHOP and GRP78 expression.

Results: Chondrocytes stimulated with sublethal cyclic compressive stress demonstrated rapid increase over 1–7 days in both GRP78 and CHOP. In human knee OA, CHOP and GRP78 expression were markedly increased in cartilage sections from the uncovered tibial plateau and weight-bearing femoral condyle areas, especially in chondrocyte clusters in the middle and deep zones. Isolated human knee chondrocytes from grades 2–3 OA demonstrated increased XBP1s. However, a dysfunctional UPR in human knee OA was observed by microarray data showing flat GRP78 and CHOP expression in OA vs. normal cartilage chondrocytes, in the context of increased chaperone, protein disulfide isomerase (PDI), and chondrocyte hypertrophy marker (type X collagen, osteopontin) expression. CHOP and GRP78 expression also became steadily and markedly decreased with aging in normal mouse knees, and UPR activation with tunicamycin (UPR activator) in addition to mechanical stress resulted in 2-fold increased GAG (p < 0.01) and 3-fold increased NO (p < 0.001) release beyond that demonstrated with compression alone.

Conclusions: We observed increased expression of the core UPR mediators GRP78 and CHOP in mechanically stressed chondrocytes in vitro, in human cartilage zones most affected by mechanical stress, and in chondrocyte clusters in late stage OA. However, there was flat RNA with GRP78 and CHOP in human OA chondrocytes. Moreover, CHOP and GRP78 were significantly depressed in aging mouse knee cartilages and sustained activation of the UPR via tunicamycin promoted chondrocyte catabolic responses. Taken together, these results indicate that the UPR is robustly activated by biomechanical stress, but dysregulation of the UPR in aging or sustained UPR activation with unresolved stress may promote OA progression.

262 RESVERATROL DOES NOT IMPROVE CARTILAGE FORMATION BY OSTEOARTHRITIC CHONDROCYTES

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Purpose: Resveratrol is a phytoalexin stilbene produced naturally by plants including red grapes and peanuts. Many studies have established that resveratrol can exert a broad range of biological activities including suppression of inflammatory signaling and activation of sirtuins. It has been shown that resveratrol suppresses IL-1β-induced inflammatory signaling in chondrocytes and it prevented cartilage damage in experimentally induced osteoarthritis (OA) (by anterior cruciate ligament transaction) in rabbits. The aim of this study was to investigate the effects of resveratrol on cartilage formation by osteoarthritic chondrocytes.

Methods: OA chondrocytes were isolated from articular cartilage obtained from patients undergoing knee arthroplasty. At passage 2, chondrocytes were seeded at high density (1.26×10⁶ cells per cm²) on type II collagen-coated culture inserts in a 96-wells transwell system. The cells were precultured for five days without any treatment, after which, they were either or not supplemented with 50 μM resveratrol (RSV) and/or 20 mM nicotinamide (NAM) to inhibit sirtuin activation. At day 7, 5 ng/ml IL-1β was added after which the cells were cultured for another 3 days. Cytotoxicity was determined by measuring LDH release into the culture medium, cartilage turnover by glycosaminoglycan (GAG) content and release into the culture medium by the DMMB assay and DNA content using a Picogreen assay.

Results: Treatment with IL-1β increased the amount of LDH released into the culture medium. Co-treatment of IL-1β with RSV suppressed this increase, while co-treatment of IL-1β with RSV and NAM showed no suppression. Treatment with RSV increased the total production of GAG compared to baseline (P < 0.01), however, 12% was incorporated in the matrix and 82% was released into the medium. Treatment with NAM resulted in 23% of the GAGs incorporated in the matrix. Treatment with IL-1β decreased GAG production and GAG incorporation (18%; P < 0.05). Co-treatment of IL-1β with RSV increased both the total GAG production and the incorporation (20%) compared to IL-1β treatment alone (P < 0.01). Co-treatment with IL-1β, RSV and NAM had no effect on total GAG production compared to baseline, but decreased GAG incorporation to 19% (P < 0.05).
whether chondrons from affected cartilage also produce better cartilage compared to chondrocytes. In addition, the influence of harvest location in the knee was investigated.

**Methods:** Cartilage tissue was harvested from goats in which the medial cartilage surface of the femur of the right knee was grooved and the left knee was not operated. Harvest locations were the medial and lateral condyles on both the tibial and femoral plateau of both knees. Part of the tissue was used for chondrocyte isolation and the other part for chondron isolation. Directly after isolation, the cells and chondrons were seeded at high density \((1.26 \pm 0.06) \times 10^6\) cells per cm² on collagen-coated culture inserts in a 24-wells transwell system. After 4 weeks of culture, glycosaminoglycan (GAG), collagen and DNA content were determined using a DMMB, hydroxyproline and Picogreen assay, respectively.

**Results:** Cartilage regenerated from chondrons isolated from the grooved surface, the medial femoral condyle of the right knee, contained less GAGs and collagen compared to tissue generated from chondrons from the same location in the nonoperated knee \((P < 0.01)\). Chondrons from the lateral femoral condyle of the grooved knee produced more GAGs and collagen compared to grooved chondrons \((P < 0.01)\), but less compared to chondrons from the lateral femoral condyle of the nonoperated knee \((P < 0.01)\). Chondrons from both lateral condyles produced more GAGs and collagen compared to the medial condyle chondrons \((P < 0.01)\). The same pattern was shown for chondrons isolated from the tibial locations, but the amount of matrix produced was lower compared to the femoral locations.

When chondrocytes were used for in vitro cartilage regeneration, less pronounced differences were noted. Collagen production by chondrocytes isolated from the grooved medial femur was lower compared to chondrocytes from the lateral femoral condyle of the right knee and from the medial femoral condyle of the nonoperated knee \((P < 0.01)\). Moreover, for all locations, chondrocytes produced less GAGs and collagen compared to chondrons isolated from the same locations \((P < 0.001)\).

**Conclusions:** Chondrons performed better in cartilage production compared to chondrocytes. Moreover their regenerative capacity clearly reflects whether they were isolated from affected or healthy cartilage. The femoral lateral condyle seems the optimal harvest site for chondrons reflecting whether they were isolated from affected or healthy cartilage. Moreover their regenerative capacity clearly indicates whether they were isolated from affected or healthy cartilage.

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**Purpose:** Chondrocyte apoptosis contributes to the disruption of cartilage integrity in osteoarthritics (OA). Recently, it has been suggested that activation of volume-sensitive CI− current \((I_{Cl,vol})\) 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 \(I_{Cl,vol}\) in rabbit articular chondrocytes using whole-cell patch-clamp technique.

**Methods:** Preparation of chondrocytes: Rabbit cartilages were collected from the knee joint, the glenohumeral joint or the hip joint of male animals weighing 2.5 to 3.0 kg. The cartilage was dissected into slices and then cultured in DMEM for 1–3 days. On the day of experiments, chondrocytes were isolated by enzymatic digestion. Patch-clamp recording: For the membrane current recording, a whole-cell patch-clamp method was adapted to the freshly isolated cells. To record \(I_{Cl,vol}\) with minimum contamination of other ionic currents, \(K^+\) channel, \(Ca^{2+}\) channel and \(Na^+\) channel were blocked appropriately. Furthermore, stretch-activated nonelective cationic current was inhibited by external application of \(Cd^{2+}\). Osmolarity of experimental solution was adjusted with mannitol.

**Microscopy and image analysis:** Isolated chondrocytes were allowed to settle onto the experimental chamber mounted on an inverted microscope. The chamber was continuously perfused with bathing solutions at the rate of 2–3 ml min⁻¹. Microscope images of chondrocytes were recorded with a CCD digital camera (DS-FI1, Nikon, Tokyo, Japan) every 1 min, and the area of the cell image was measured using Image-J public domain software (NIH, Bethesda, MD, USA).

**Results:** Exposure of isolated chondrocytes to doxorubicin \((1 \mu M)\) resulted in an obvious increase in the membrane CI− conductance without any appreciable change in cell size. The doxorubicin-evoked CI− current exhibited many properties almost identical with \(I_{Cl,vol}\) phenotype, including outward rectification, prominent inactivation at large positive potential \(> +50\) mV, inhibition by hyperosmotic cell shrinkage, and sensitivity to \(I_{Cl,vol}\) blockers, arachidonic acid \((10 \mu M)\) or DCPIB \((20 \mu M)\).

Pretreatment of cells with 17β-estradiol \((1 \mathrm{~nm})\) dramatically inhibited the \(I_{Cl,vol}\) activation by doxorubicin as well as subsequent apoptotic events such as AVD and elevation of caspase-3/7 activity. It was unlikely that 17β-estradiol produced a direct action on \(I_{Cl,vol}\), because it had little effect on \(I_{Cl,vol}\) activated by hypotonic cell swelling. On the other hand, the effect of 17β-estradiol was significantly attenuated by an estrogen receptor blockerICI182780 \((10 \mu M)\).

**Conclusions:** These results suggested that 17β-estradiol may prevent the doxorubicin-induced apoptosis by interfering the activation of \(I_{Cl,vol}\) in rabbit articular chondrocytes.

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**Purpose:** Synovial inflammation involves the induction of catabolic cytokines such as interleukin-1 (IL-1) that influence both bone and cartilage homeostasis and play a significant role in osteoarthritis. A previous study, using enzymatically isolated chondrocytes and cartilage explants cut clear of the underlying bone, demonstrated transient increase in intracellular calcium \(([[Ca^{2+}]]_i))\) after exposure to IL-1α. This study involved disruption of chondrocyte/matrix interactions resulting from digestion or bone removal. Undisturbed attachment to the extracellular matrix has been shown to attenuate the \([Ca^{2+}]_i\) response of chondrocytes to osmotic stimuli – 20% of cells responding in undisturbed cartilage compared to 65% of isolated cells. Based on these data we hypothesized that IL-1α would elicit a significant \([Ca^{2+}]_i\) response in chondrocytes on intact murine femora and that the percentage of chondrocytes responding would be lower in this ex vivo preparation compared to previous studies using explants or cells in vitro.

**Methods:** All animal procedures were approved by the University of Calgary Animal Care Committee. Skeletally mature mice (mass=24±4g (mean ± sd)) were sacrificed and the femora isolated. The femora were