ARTICULAR LEVELS OF ADIPONECTIN ARE NOT CHONDROPROTECTIVE IN 3D CULTURES OF HUMAN CHONDROCYTES

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Purpose: Adipokines are found in synovial fluid of OA patients and circulating levels of adiponectin (Apn) are reduced in obesity. As obese patients are at increasing risk of developing osteoarthritis (OA), even in non-weight bearing joints, we tested the hypothesis that low (physiological) concentrations of Apn could impair chondrocytes functions towards an “OA-like” phenotype.

Methods: Firstly, the expression of Apn and Apn receptors (AdipoR1) was characterized by quantitative RT-PCR and western blotting. Secondly, a dose-ranging comparative study of physiological concentrations (0.2, 1 or 5µg/ml) of globular (gApn) and full length (fApn) forms of adiponectin was performed on chondrocytes cultured in alginate beads. The parameters measured were: proteoglycans (PGs) synthesis (by radiolabelled sulfate incorporation), pro-inflammatory mediators release (NOx by Griess method, PGE2 and TNF-α by ELISA) and MMP-13 activity (by fluorimetric assay). Thirdly, in co-stimulation experiments, the abilities of gApn or fApn to modify chondrocytes responses to the pro-catabolic factor, IL-1beta (10ng/ml) or the pro-anabolic factor, IGF-1 (50ng/ml) was studied.

Results: We demonstrated that chondrocytes expressed Apn (mRNA & protein) in monolayers or alginate beads as well as AdipoR1 (mRNA & Protein) and AdipoR2 (mRNA). We found that, in the concentration range tested, gApn was inactive on all parameters except a weak inhibitory effect on MMP-13 at 5µg/ml. In contrast, the highest concentration (5µg/ml) of fApn was able to decrease PGs synthesis by 27%, to induce NO and PGE2 release, and to increase MMP-13 activity. A weak effect was seen for lower concentrations of fApn on PGs synthesis and MMP-13 activity. Neither gApn nor fApn reduced the stimulating effect of IGF-1 on PGs synthesis, but fApn weakly potentiated the inducing effect of IL-1beta on PGE2 release and MMP-13 activity.

Conclusions: This preliminary study shows that, in human chondrocytes: i) gApn was less active than fApn; ii) fApn was active in the upper range of Apn joint levels found in OA patients; iii) fApn failed to protect from the deleterious effect of IL-1. Our data do not support the meaning that Apn could be chondroprotective OA nor that a reduced articular level could be deleterious for cartilage.

CHONDROCYTE APOPTOSIS IN ARTICULAR CARTILAGE: HOW LONG DOES IT TAKE?

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Purpose: Osteoarthritis (OA) is a prevalent degenerative disorder, especially in ageing individuals. Loss of articular cartilage (AC) is a key feature of the disease and recent studies suggest that a reduction in cell viability may be a trigger for both initiation and progression of AC damage in OA. Our previous studies have shown that hypocellularity in OA cartilage can be explained by apoptosis which is on average 3 times higher in OA cartilage compared to normal (Sharif et al 2004). The aim of this study was to determine the time course for chondrocyte apoptosis in equine AC.

Methods: Equine AC obtained from the metatarsophalangeal (MTP) joint of 9 horses were incubated with tumour necrosis factor-alpha (TNF-α) and Actinomycin-D (ActD) for 0, 6, 12, 18, 24 and 48 hours. At each time point cartilage was assessed for cell viability using fluorescein diacetate (stains live cells green) and propidium iodide (stains dead cells red) and apoptosis using indirect immunohistochemistry for active caspase-3 expression.

Results: Cell viability was 89.9 ± 1.3% (Mean ± SEM) at baseline and decreased linearly to 69.9 ± 5.9% after 24 hours. Overall levels of cell death after 18 (P<0.05), 24 and 48 hours (P<0.01) were found to be statistically significant. Cell death by apoptosis (expression of caspase-3) also reached levels of significance after these time points. Apoptosis increased linearly from 27.1 ± 1.3% at baseline to 58.8 ± 5.1% after 24 hours. In addition, a significant positive correlation (r = 0.53, p<0.001) was found between the percentages of dead cells and apoptotic cells.

Conclusions: Our data demonstrated that TNF-α plus ActD treatment of equine AC over 48 hours results in peak apoptosis at 24 hours, and that apoptosis contributes significantly to the overall increase in cell death over the 48 hours. These findings support the role of chondrocyte apoptosis in the initiation of AC damage in OA.

INVESTIGATING SUPEROXIDE DISMUTASE EXPRESSION IN CARTILAGE AND A POTENTIAL ROLE IN OSTEOARTHRITIS

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Purpose: To characterise the expression of the superoxide dismutase (SOD) family of antioxidant enzymes in normal and osteoarthritic cartilage and investigate their potential role in the development of osteoarthritis (OA).

Methods: SOD gene expression was determined in RNA samples extracted from the cartilage of 12 normal fracture (NOF) and 12 OA hips using real time PCR. Immunohistochemistry was then used to assess the levels of SOD2 protein expression in NOF and OA hip cartilage sections. Histochemical stains for the electron transport chain proteins cytochrome c oxidase (COX) and succinate dehydrogenase (SDH), which are mitochondrially and nuclear encoded respectively, were used to identify any OA chondrocytes exhibiting mitochondrial dysfunction as a potential consequence of damage by reactive oxygen species (ROS). The mitochondrial genome of individual COX deficient cells was amplified by PCR and the mutational status determined by DNA sequencing.

Results: The expression of all SOD genes was significantly downregulated at the mRNA level. The downregulation of SOD2 appeared to be the most dramatic and this has been confirmed at the protein level. COX-SDH histochemistry identified a proportion of OA chondrocytes having deficient expression of the mitochondrially encoded COX enzyme.

Conclusions: SOD2 expression has been shown to be downregulated in OA cartilage compared to NOF cartilage. Subsequently, OA chondrocytes which contain potentially dysfunctional mitochondria have been identified histochemically and further work is being carried out to identify whether this is a consequence of increased levels of mitochondrial DNA (mtDNA) mutation. Mitochondrial dysfunction and mtDNA mutation may arise as a result of the downregulation of SOD2 expression and consequent increased levels of ROS. Future work will examine mtDNA mutation rates between age-matched OA and normal chondrocytes. We propose that re-instigation of normal levels of SOD2 expression, or antioxidant therapy, will reduce oxidative stress and damage seen in OA and may represent a potential therapy. In certain