Conclusions: The results indicate a reduction of H₂S synthesis as a critical feature involved in hyperglucidic-mediated dysregulation of articular chondrocytes. The impairment of H₂S signalling could participate in the mechanisms underlying the predisposition to OA development in diabetic individuals and may open new opportunities for treating patients with a diabetes-related OA phenotype.

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SAT0060 PROTEOMIC ANALYSIS OF OSTEOBLASTS SECRETOME PROVIDES NEW INSIGHTS IN MECHANISMS UNDERLYING OSTEOARTHRITIS SUBCHONDRAL BONE SCLEROSIS

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Background: Osteoarthritis (OA) is characterised by cartilage degradation but also by other joint tissues modification like subchondral bone sclerosis

Objectives: In this study, we used a proteomic approach to compare secretome of osteoblasts isolated from sclerotic (SC) or non sclerotic (NSC) area of OA subchondral bone

Methods: Secretome was analysed using differential quantitative and relative label free analysis on nanoUPLC G2 HDMS system. mRNA of the more differentially secreted proteins were then quantified by RT-PCR and the most relevant proteins identified using western-blotting and immunoassays

Results: 175 proteins were identified in NSC osteoblasts secretome. Compared to NSC osteoblasts secretome, 13 proteins were significantly less secreted (Osteomodulin, CSF-1, IGFBP5, VCAM-1, IGF2, 78 kDa glucose-regulated

protein, versican, calumenin, IGFBP2, thrombospondin-4, periostin, reticulocalbin 1 and osteonectin), and 12 proteins significantly more secreted by SC osteoblasts (CHI3L1, fibulin-3, SERPINE2, IGFBP6, SH3BGRL3, SERPINE1, reticulocalbin3, alpha-2-HS-glycoprotein, TIMP-2, IGFBP3, TIMP-1, SERPINF1). Similar changes in periostin, osteomodulin, SERPINE1, IGFBP6, fibulin-3 and CHI3L1 mRNA levels were observed. Finally, osteomodulin and fibulin-3 specific sequences were quantified by western blot and immunoassays in serum and osteoblasts conditioned culture supernatants.

Conclusions: We highlighted some proteins differentially secreted by NSC and SC osteoblasts of OA subchondral bone sclerosis. These changes contribute to explain some features observed in OA subchondral bone, like the increase of bone remodelling or abnormalities in bone matrix mineralization. Among identified proteins, osteomodulin was found decreased and fibulin-3 increased in serum of OA patients. These findings suggest that osteomodulin and fibulin-3 fragments could be biomarkers to monitor early changes in subchondral bone metabolism in OA.

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SAT0061 TARGETING ACTIVATED SYNOVIAL FIBROBLASTS USING PHOTODYNAMIC THERAPY IN RHEUMATOID ARTHRITIS

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Background: Activated synovial fibroblasts (SF) play an important role in the pathogenesis of rheumatoid arthritis (RA). They contribute to the pro-inflammatory environment in the joint as well as to the degradation of cartilage. Depleting SF could ameliorate both the symptoms of joint inflammation and degradation in RA. SF are characterised by the expression of Fibroblast Activation Protein (FAP). Here, we investigated the potential of photodynamic therapy (PDT) targeting FAP to selectively induce cell death in these cells as well as in synovial tissue from RA patients. In PDT, a light-sensitive molecule is delivered to a target cell and activated with light of a specific wavelength. This causes cell death through the production of reactive oxygen species.

Methods: The anti-FAP antibody 28 H1 was conjugated with the photosensitizer IRDye700DX (28 H1–700DX). *In vitro* PDT assays were performed with 3 T3 fibroblasts stably transfected with FAP. 3T3-FAP cells were incubated with 28 H1–700DX or a control conjugate for 4 hours, and exposed to varying 690 nm light exposures. Subsequently, cell viability was measured using the CellTiter-Glo assay. For *ex vivo* evaluation of PDT efficiency, human RA synovial tissue obtained after joint replacement surgery was processed into standardised 6 mm biopsies and used for FAP-based PDT. The biopsies were incubated with 28 H1–700DX for 4 hours, subjected to 52 J/cm² light exposure and fixed in formalin after 1 hour. Tissue was then embedded in paraffin and stained for the presence of gH2AX and caspase 3 as indicators of DNA double-strand breaks and early apoptosis on sequential slides. The presence of FAP was also determined on subsequent slides.

Results: The effect of PDT was optimal at 13.7 J/cm² light exposure to 3T3-FAP cells incubated with 6.67 pM 28 H1–700DX, which dramatically reduced cell viability with 89.27%±2.48 compared to control ($p < 0.001$). No cell death was observed with the control 700DX-conjugate ($p = 0.16$).

In the PDT experiment on human RA synovial biopsies, the groups incubated with 28 H1–700DX and exposed to light showed apparent cell death in the synovial tissue as evidenced by the positive staining of both the gH2AX and caspase 3 markers (figure J and K). Staining of these markers co-localised with areas of high FAP staining (figure L). This was not the case in the control samples that were not exposed to either 28 H1–700DX and/or light (figure A – I). All biopsies did show FAP staining indicating that the cell death was only achieved when the biopsies were exposed to both the antibody and the light.

Conclusions: We have demonstrated fibroblast-specific cell death by targeted PDT using 700DX-conjugated 28 H1. Furthermore, we demonstrated that PDT also induces cell death of FAP-positive cells in synovial tissue from RA patients, suggesting FAP-targeted PDT as a promising new tool in treating RA.

Disclosure of Interest: None declared

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SAT0062

METABOLOMICS AND METABOLIC FUNCTION ANALYSIS OF THE SECRETOME OF ARTICULAR CARTILAGE AND CHONDROCYTES IN RESPONSE TO PRO-INFLAMMATORY CYTOKINES

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Background: Chondrocytes rely primarily on glycolysis to meet their energy requirements, but can support cell survival and matrix synthesis during periods of nutrient stress by enhancing glycolysis with mitochondrial respiration. Accessing this 'spare respiratory capacity' requires optimal mitochondrial function. Impaired mitochondrial function is implicated in osteoarthritis (OA). Metabolic adaptation is evident in early-stage OA, however cartilage from late-stage disease does not seem to have this flexibility. A deeper understanding of these complex metabolic pathways may identify new markers of disease stage, and support therapeutic strategies for treating OA.

Objectives: Metabolomics has the potential to reveal pathological pathways and identify novel biomarkers. The aim was to identify metabolic processes involved in early stage disease by analysis of metabolites and metabolic function in pro-inflammatory models of cartilage degradation.

Methods: Macroscopically normal articular cartilage was obtained from equine and bovine metacarpophalangeal joints. Equine cartilage explants (n=6), and primary chondrocytes seeded at 105,000/cm² (n=4), were cultured for 7 days in serum-free DMEM (Gibco) with or without 10 ng/ml equine interleukin-1 β (IL-1 β) and 10 ng/ml tumour necrosis factor- α (TNF- α). Secretome metabolite levels were measured using AbsoluteIDQ p180 targeted metabolomics kit (Biocrates), with Waters Xevo TQ-S mass spectrometer coupled to an Acquity UPLC system. PCA and OPLS-DA were performed using SIMCA-P v12.0 software. Metabolic function of primary equine (n=9) and bovine chondrocytes (n=3) was determined using Seahorse XFp and XF24 analyzers. Cells were treated with species-specific 10 ng/ml IL-1 β and/or 10 ng/ml TNF- α for 18 hour, and metabolically challenged with the Mito Stress Test. Metabolite levels, and oxygen consumption rates, were normalised to total cell protein, and values analysed by ANOVA with Tukey's multiple comparison post-tests.

Results: Cytokine treatment decreased proline, ornithine and alpha-aminoadipic acid (p<0.0001) in explant secretome. Citrulline increased with cytokine treatment (p<0.0001) and glutamate, present in DMEM, was also elevated (p<0.0001). Metabolomic analysis of chondrocyte secretome showed that glutamine decreased (p<0.02) with cytokine treatment whereas citrulline was elevated (p<0.003). Metabolic analysis showed that cytokine treatment reduced basal respiration and negated spare respiratory capacity in chondrocytes (p<0.01), and the effect was due to IL-1 β alone.

Conclusions: Explant metabolites which decreased with cytokine treatment are all downstream of glutamate. With elevated glutamate, this suggests that cytokines inhibit glutamate uptake and metabolism. Elevated citrulline in cell and explant models may be attributed to disruption of the urea cycle via induction of nitric oxide synthase. IL-1 β alone negated spare respiratory capacity, and chondrocytes remained glycolytic. In conclusion, cytokines disrupt glutamate and citrulline metabolism, normally tightly regulated mitochondrial pathways, and IL-1 β alone is responsible for the metabolic switch. These metabolic pathways could provide markers of early-stage inflammatory disease.

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SAT0063

EFFECTS OF INTRA-ARTICULAR INJECTED DIACEREIN-LOADED NANOPARTICLES ON JOINT IN RAT MODEL OF OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is a major health problem in recent years, but the current medical treatment is mainly symptom control and joint disability improvement. Diacerein (DIA) reduces the level of interleukin (IL)–1 receptor on the surface of chondrocytes, alleviates the pain, and prevents the structural degradation of the joint tissue. However, DIA has the side effects such as diarrhoea and urine discoloration when taken orally. To increase bioavailability and reduce systemic side effects, it is preferable to inject DIA directly into the joints. Nanoparticulate drug carriers have been attempted with the advances in drug delivery systems.

Objectives: We investigated that DIA-loaded nanoparticles (DIA/NPs) can efficiently inhibit the inflammatory reaction in synoviocytes stimulated by lipopolysaccharide *in vitro* and alleviate both inflammation and cartilage degeneration in monosodium iodoacetate (MIA)-induced OA rat model *in vivo*.

Methods: DIA/NPs were fabricated by water/oil/water emulsion method. *In vitro*, the mRNA levels of pro-inflammatory cytokines (IL-1, IL-6, MMP-3, MMP-13, COX-2, ADAMTS-5, and TNF- α) were measured at 1 st and 3rd day after the administration of NPs only and DIA/NPs to synoviocytes using real-time PCR. MIA was intra-articular injected through the infrapatellar ligament of the rats' knee to induce OA. The rats were randomly divided into the six treatment groups: ¹ control, ² MIA, ³ MIA and NPs, ⁴ MIA and DIA(1%)/NPs, ⁵ MIA and DIA(5%)/NPs, and ⁶ MIA and DIA(5%) solution injection. After NPs, DIA(1%)/NPs, DIA(5%)/NPs, and DIA(5%) solution were injected, at 8th week, the rats were sacrificed to evaluate the plain radiographic and micro-computed tomography (micro-CT), histological study, and pro-inflammatory cytokines expression.

Results: The mRNA expression levels for pro-inflammatory cytokines in cells seeded with DIA(5%)/NPs and DIA(5%) solution were significantly lower than those in cells seeded with NPs at 1 st and 3rd day. Moreover, the mRNA levels of pro-inflammatory cytokines in cells injected with DIA(5%) solution decreased much greater than in cells injected with DIA(5%)/NPs. The rats injected DIA(5%)/NPs showed the least amount of cartilage and bone damage on plain radiography and micro-CT and had the less cartilage loss and the bony erosions on microscopic observation. The pro-inflammatory cytokines expression was lowest in the rats injected DIA(5%)/NPs, followed by NPs, DIA(1%)/NPs, and DIA(5%) solution.

Abstract SAT0063 – Figure 1. The macroscopic observations for 9 weeks after the MIA injection and for 8 weeks after treated with NPs, DIA (1%)/NPs, DIA (5%)/NPs, or DIA (5%) solution injections (arrows indicate erosions).

Conclusions: DIA(5%)/NPs are a promising therapeutic material to control the symptoms and prevent the progression of OA.