SAT0057

THE EFFECT OF EXOSOMES FROM BONE MARROW MESENCHYMAL STEM CELLS ON OSTEOARTHRITISH. Zhu, J. Ji, T. Fu, J. Yang, Z. Gu. *Department of Rheumatology, Affiliated Hospital of Nantong University, Nantong, China*

Background: Mesenchymal stem cells (MSCs) exert chondroprotective effects in clinical models of osteoarthritis (OA), but the exact mechanisms were still unclear. Exosomes that serve as carriers of genetic information have been implicated in many diseases and are known to participate in many physiological processes.

Objectives: Here, we investigate the therapeutic potential of exosomes from human bone marrow MSCs in alleviating OA and explore the mechanism.

Methods: Exosomes were harvested from conditioned culture media of BM-MSCs by a sequential centrifugation process. The anterior cruciate ligament transection and destabilisation of the medial meniscus (DMM) surgery were performed on the knee joints of SD female rat as an OA model. After four weeks, the animals were followed by intra-articular injection of either BM-MSCs or their exosomes every week for four weeks. Cartilage destruction, matrix degradation and subchondral bone changes were evaluated with histological staining and micro-CT at the post-surgery 8 weeks. Primary human chondrocytes treated with IL-1 β were used as an *in vitro* model to evaluate the effects of exosomes for 24 hours.

Results: We found that intra-articular injection of BM-MSCs and BM-MSCs derived exosomes improve cartilage destruction and subchondral bone remodelling in ACLT+DMM model. BM-MSCs and exosomes equally protected rat from joint damage. These exosomes maintained the chondrocyte matrix by increasing collagen type II synthesis and decreasing ADAMTS5, MMP13 and Col II expression in the presence of IL-1 β *in vitro*. In addition, BM-MSCs derived exosomes were also shown to protect chondrocytes from apoptosis and senescence.

Conclusions: The exosomes from BM-MSCs exert a beneficial therapeutic effect on OA by not only repairing the degenerative cartilage, but also improving subchondral bone remodelling, which in turn provides a new target for OA drug and drug-delivery system development.

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SAT0058

ADALIMUMAB:TNF COMPLEXES ARE CLEARED MORE EFFICIENTLY BY HUMAN OSTEOCLASTS THAN THOSE WITH ETANERCEPT THROUGH FCG-RECEPTOR BINDING AND INTERNALISATIONB.P. Harvey, J. Cohen-Solal, Z. Kaymakalan. *Biologics, Abbvie Bioresearch Center, Worcester, USA*

Background: TNF-alpha (TNFa) has been shown to contribute to osteoclastogenesis (OCgenesis) independently and in conjunction with M-CSF or RANKL, two key cytokines involved in osteoclast (OC) development. We have previously demonstrated that TNF enhances the kinetics of RANKL-induced human OCgenesis and that its effects are mitigated more effectively by the anti-TNF biologic adalimumab (ADA) as compared to etanercept (ETN).

Objectives: To determine whether Fc-gamma receptor (FcGR)-mediated internalisation of the biologic:TNF complexes is a contributing mechanism responsible for the difference in effectiveness between ADA and ETN in preventing TNF-enhanced OCgenesis.

Methods: TNF biologics [ADA and ETN] alone or in preformed complexes with TNFa at 50:1 molar ratio were tested for FcGR binding by flow cytometry using CHO stably transfected with human FcGRs (FcGR1, FcGR1A, -RIIB, -RIIC, FcGR1IIA and -RIIIB). FcGR expression and binding of preformed biologic:TNF complexes at 10:1 ratio $\pm$ FcGR blocking antibodies to primary human OC precursors (OCP) was evaluated by flow cytometry. FcGR-mediated internalisation was

assessed by monitoring a reduction in OC survival in response to preformed biologic:TNF complexes (25:1 ratio) bound with saporin (ZAP), a ribosome-inactivating toxin, as anti-human Fc IgG Fab conjugate $\pm$ FcGR blocking antibodies.

Results: The binding study to CHO (human FcGRs) cell lines showed that monomeric ADA and ETN bind similarly to FcGR (highly on high affinity FcGR and loosely on low affinity FcGRs) while preformed biologics: TNF complexes bind differently. ADA:TNF complexes bind to low affinity FcGR, whereas ETN:TNF keep a monomeric binding profile with no gain of binding to low affinity FcGR. OCP were found to express mostly FcGR1I early in development with predominant binding of only ADA:TNF, not ETN:TNF, to this FcGR with additional binding to undefined receptor(s). Despite subsequent increases in FcGR1 and R1II later on, ADA:TNF still preferentially bound to FcGR1I on the matured OCP with minimal binding to R1II, whereas ETN:TNF binding was observed only to FcGR1. Exposure of OCP to ADA:TNF:ZAP(toxin) complexes led to a significant reduction (4-fold) in mature OC due to complex internalisation as compared to human IgG:ZAP + TNF conditions that was partially rescued only with the addition of FcGR1I blocking antibody. Interestingly, a 1.5-fold reduction in mature OC was observed with ETN:TNF:ZAP.

Conclusions: Our *in vitro* findings demonstrate that human OCP can bind and internalise ADA:TNF complexes more efficiently than ETN:TNF complexes. In addition, this process is partially mediated through FcGR1I. Clearance of the ADA:TNF complexes may help reduce exposure of the OCP to localised TNF by removing TNF more effectively in the joint environment. Additional *in vivo* analysis need to be done to verify these *in vitro* findings.

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SAT0059

IMPAIRMENT IN HYDROGEN SULFIDE SYNTHESIS IN OSTEOARTHRITIC CHONDROCYTES FROM DIABETIC PATIENT AND UNDER A HIGH GLUCOSE STRESSC. Vaamonde-Garcia^{1,2}, F. Blanco², R. Meijide-Falide¹. ¹*Biological science, Medicine and Physiotherapy, University of A Coruña;* ²*Rheumatology, INIBIC-CHUAC, A Coruña, Spain*

Background: A growing number of findings support the hypothesis that type 2 diabetes is an independent risk factor of osteoarthritis (OA). However, the mechanisms underlying the connexion between both diseases remain unclear. Hydrogen sulfide (H₂S) plays an important role in the pathogenesis of diabetes and its complications. In relation, we and other authors have observed a protective impact of H₂S induction on activation of pathological pathways in the chondrocyte.¹

Objectives: In this study we examined the modulation of H₂S levels in osteoarthritic chondrocytes from diabetic (DB) or non-diabetic (non-DB) patients subjected or under glucose stress, in order to elucidate whether impairment in H₂S-mediated signalling could participate in the establishment of diabetes-related OA.

Methods: Chondrocytes were isolated from OA cartilage of diabetic (DB) or non diabetic (non-DB) patients. T/C28a2 and primary human chondrocytes were stimulated w/o IL-1 β (5 ng/mL) under a normal (5.5 mM; NG) or a high (25 mM; HG) glucose environment. Gene and protein expression of enzymes involved in H₂S synthesis (cystathionine γ -lyase [CSE], cystathionine β -synthase [CBS], and 3-mercaptopyruvate sulfurtransferase [3-MT]) and HO-1 were assessed by RT-qPCR and WB, respectively. To determine the involvement of H₂S in catabolic pathways activated by HG in chondrocytes, NaSH and GYY 4137 (500 μ M), a fast and slow-releasing H₂S donor respectively, were employed.

Results: Fresh isolated chondrocytes from OA cartilage of diabetic patients showed lower levels of H₂S synthesising enzymes (CSE, CBS and 3-MT) than those of non-DB patients (figure 1). In relation, chondrocytes T/C28a2 exposed to HG stress expressed lower mRNA and protein levels of these 3 enzymes after 3 days of incubation compared to those incubated in NG conditions (0.41-fold and 0.83-fold [CSE], 0.42-fold and 0.66-fold [CBS], and 0.52-fold and 0.79-fold [3-MT] for mRNA and protein expression, respectively; n=6, p<0.05). IL-1 β also attenuated the gene and protein expression of CBS elicited by chondrocytes incubated in NG (0.47-fold and 0.86-fold, respectively; n=6, p<0.05). Additionally, the expression of pro-inflammatory chemokine IL-8 induced by IL-1 β was significantly higher in chondrocytes under HG than NG condition (6-fold; n=5, p<0.05); whereas protein levels of heme oxygenase 1, an anti-inflammatory enzyme, were reduced in HG exposed chondrocytes (0.77-fold; n=6, p<0.05). GYY 4137 and NaSH co-treatment recovered HO-1 expression and reduced IL-8 levels in chondrocytes under IL-1 β +HG conditions. Furthermore, similar results were registered in primary human chondrocytes from OA cartilage.