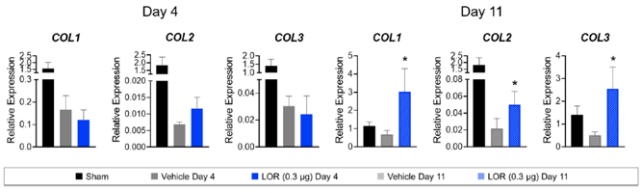

Figure 1. LOR increased collagen gene expression in vivo. A single IA injection of monosodium iodoacetate (MIA; 3 mg) was immediately followed by a single IA injection of LOR (0.3 µg) or vehicle at 10 weeks of age. Knees were harvested on Days 1, 4, and 11 after injection and menisci were isolated. Gene expression was measured by qRT-PCR. N=3, Mean ± SEM, *P<0.05, one-way ANOVA

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Disclosure of Interests: Tim Seo Shareholder of: Samumed, LLC, Employee of: Samumed, LLC, Vishal Deshmukh Shareholder of: Samumed, LLC, Employee of: Samumed, LLC, Yusuf Yazici Shareholder of: Samumed, LLC, Employee of: Samumed, LLC
DOI: 10.1136/annrheumdis-2021-eular.611

POS0373 LIRAGLUTIDE HAS POTENT ANTI-INFLAMMATORY AND ANTI-CATABOLIC IN VITRO ACTIVITIES IN OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is an age-related joint disease which provokes chronic pain and limits mobility. The disease progression is associated with inflammatory responses and cartilage degradation. Both chondrocytes, the only cell type present in cartilage, and macrophages from the synovium, play a major role in OA pathophysiology. Liraglutide is a Glucagon-Like Peptide-1 Receptor (GLP-1R) agonist widely prescribed for the treatment of type 2 diabetes. Interestingly, anti-inflammatory properties of the GLP-1 pathway have been reported in various diseases outside diabetes.
Objectives: We evaluated the anti-inflammatory and anti-catabolic effects of Liraglutide in two *in vitro* models relevant to OA by evaluating surrogate markers of inflammation, cartilage matrix proteolysis and differentiation.
Methods: Lipopolysaccharide (LPS)-stimulated murine Raw 264.7 macrophages were treated with 10 concentrations (6.6nM-3.4µM) of Liraglutide for 24h. Anti-inflammatory activity was evaluated by the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) using Griess reaction and ELISA, respectively. Interleukin 1β (IL-1β)-stimulated mouse articular chondrocytes were treated with Liraglutide (6.6nM-3.4µM) for 24h. Production of IL-6, matrix metalloproteinase-3 (MMP-3) and glycosaminoglycans (GAG) was measured by ELISA and GAG assay, respectively. RTqPCR analyses were performed with three selected concentrations of Liraglutide (13.3nM, 53.1nM and 1.7µM) on both cell types to assess the expression of a panel of genes related to inflammation (IL-6, TNF, iNOS), M1/M2 macrophage phenotype (MCP-1, CD38, ERG-2), catabolism (MMP-13, ADAMTS-5) and differentiation (Sox9, Col2a1, Acan).
Results: Liraglutide induced a dose-dependent inhibition of the LPS-induced production of NO (IC_{50}=45nM) and PGE_2 (IC_{50}=54nM) in macrophages. Moreover, IL-6 and TNF gene expressions were significantly and dose-dependently decreased in Raw 264.7 cells treated with Liraglutide compared to LPS alone. Interestingly, there was a significant dose-dependent reduction of MCP-1 and CD38 (M1 marker) gene expression in cells treated with the 3 doses of Liraglutide compared to LPS alone while we observed a dose-dependent increase of ERG-2 (M2 marker) gene expression induced by Liraglutide. Liraglutide significantly dose-dependently reduced the IL-1β-induced release of IL-6 (IC_{50}=38nM), MMP-3 (IC_{50}=56nM) and GAG (IC_{50}=47nM) in chondrocytes. Additionally, Liraglutide treatment dose-dependently decreased the IL-1β-induced gene expression of iNOS, MMP-13 and ADAMTS-5. Finally, IL-1β decreased gene expression of Sox9, Col2a1 and Acan differentiation markers, which was rescued in a dose-dependent manner by Liraglutide (Table 1).
Conclusion: A shift in M1/M2 macrophage phenotype and the inhibition of chondrocyte expression of several mediators involved in inflammation and cartilage degradation explain, at least in part, our previous results from rodent osteoarthritis models that showed an analgesic, anti-inflammatory and anti-degradative effect of Liraglutide. The fact that Liraglutide is already safely prescribed in another indication allows us to foresee a first trial in humans in the short term.
Acknowledgements: All the people who contributed to the InOsteo project: the members of 4P-Pharma, INSERM UMRS_938 research team, SATT Lutech and Sorbonne University
Disclosure of Interests: Francis Berenbaum Consultant of: Boehringer, Bone Therapeutics, CellProthera, Expanscience, Galapagos, Gilead, GSK, Merck Sereno, MSD, Nordic, Novartis, Pfizer, Regulaxis, Roche, Sandoz, Sanofi, Servier, UCB, Peptinov, 4P Pharma, 4Moving Biotech, Grant/research support from: TRB Chemedica, Coralie Meurot Employee of: 4P-Pharma, Laure Sudre Employee of: 4P-Pharma, Keren Bismuth Employee of: 4P-Pharma, Revital Rattenbach Shareholder of: 4P-Pharma, Employee of: 4P-Pharma, Patrice Denefle Speakers bureau: 4P-Pharma, Consultant of: 4P-Pharma, Pierre Fabre, Mimetas, Employee of: 4P-Pharma, Celine Martin Employee of: 4P-Pharma, Claire Jacques: None declared
DOI: 10.1136/annrheumdis-2021-eular.1849

Table 1. Gene expression results (fold change) in Raw 264.7 murine macrophages or mouse primary articular chondrocytes

Cell type	Markers	Vehicle	Vehicle	Liraglutide (nM)		
				13.3	53.1	1700
Macrophages		**- LPS**		**+ LPS**		
	IL6	1.0±0.2*	63.6±7.1	58.8±6.5	36.3±8.4*	30.8±2.6*
	TNFα	1.0±0.2*	26.8±4.9	17.5±2.6*	9.5±1.8*	4.1±2.1*
	MCP1	1.1±0.6*	91.7±11.2	67.8±4.3*	47.3±6.1*	25.1±5.5*
	CD38	1.1±0.6*	103.5±22.3	73.2±12.3*	44.6±8.9*	17.4±3.3*
	ERG2	1.0±0.3*	0.3±0.1	1.1±0.5*	2.1±0.6*	3.1±0.2*
Chondrocytes		**- IL1β**		**+ IL1β**		
	iNOS	1.0±0.2*	47.8±17.6	34.2±15.5	18.9±8.2*	11.8±2.9*
	MMP13	1.0±0.2*	9.8±2.0	7.6±1.0	4.6±0.7*	2.5±0.4*
	ADAMTS5	1.2±0.8	2.7±0.6	2.0±0.6	1.6±0.3	1.1±0.2
	Sox9	1.0±0.1*	0.4±0.2	0.4±0.2	0.6±0.0	0.7±0.2
	Col2a1	1.0±0.2*	0.3±0.1	0.3±0.1	0.5±0.3	0.8±0.3*
	Acan	1.2±0.8*	0.2±0.1	0.1±0.0	0.5±0.1*	0.7±0.2*

* p<0.05 vs LPS or IL1β alone, n=4

POS0374 SENESCENCE DID NOT ALTER THE CHONDROPROTECTIVE EFFECT OF EXTRACELLULAR VESICLES FROM MESENCHYMAL STROMAL CELLS

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Background: Age is the most important risk factor in degenerative osteoarthritis (OA) and is associated with the accumulation of senescent cells that contribute to functional decline of joint. We previously demonstrated that extracellular vesicles (EVs) from mesenchymal stromal cells (MSCs) largely mediate the therapeutic effect of parental cells in OA.
Objectives: Here, we assessed the impact of senescence on the characteristics of EVs from adipose tissue-derived MSCs (ASC-EVs) and their properties in an in vitro model of OA
Methods: ASCs were induced to senescence using 25µM etoposide for 24 hours. Senescence was assessed by quantifying proliferation rate, SA-βGal activity, nuclear γH2AX foci number, phalloidin staining and expression of cyclin dependent kinase inhibitors (CDKI) (RT-qPCR). ASC-EVs were isolated by differential ultracentrifugation and characterized by size, concentration, total protein content, structure (cryo-TEM) and immunophenotype. In vitro OA model used chondrocytes isolated from OA patients, which were stimulated with IL1β for 48h before culture with ASCs or ASC-EVs for 7 days. Expression of chondrocytic and inflammatory markers was quantified by RT-qPCR and SASP factors were quantified by ELISA in supernatants.
Results: Senescence-induced ASCs experienced growth arrest and increase of SA-βGal staining, of p21 CDKI expression, of nuclear γH2AX foci, of stress fibers and of several SASP factors (IL6, IL8, MMP3) confirming the expression of main senescence features. Senescent ASCs produced 4-fold more EVs than healthy ASCs and senescent ASC-EVs were larger. In vitro, both healthy and senescent ASCs decreased fibrotic markers (*type III COLLAGEN*), catabolic and hypertrophic markers (*MMP3, MMP13, AP*) and increased *COX2* expression in OA chondrocytes. By contrast, healthy ASCs decreased the expression of *IL6* while senescent ASCs highly increased *IL6*. Looking at the role of ASC-EVs on OA chondrocytes, we found out that both healthy and senescent ASC-EVs were able to increase the expression of *AGG* and *type II COLLAGEN* while they decreased the expression of *MMP13, AP, type X COLLAGEN, HMOX1* and *IL6*. Finally, healthy and senescent ASC-EVs decreased the number of SA-βGal positive chondrocytes but did not impact the expression of p21 in IL1β-induced chondrocytes.
Conclusion: Our results indicated a chondroprotective effect of ASC-EVs, independently of the senescent state of parental cells and suggested that EVs might act through different mechanisms than ASCs, which warrants further investigation