THE EFFECT AND MECHANISM OF HUMAN UMBILICAL CORD MESCENHYMAL STEM-CELL DERIVED EXOSOMES ON BONE DESTRUCTION OF COLLAGEN INDUCED ARTHRITIS RATS

Jinfang Gao, Ke Xu, Gailian Zhang, Jian Han, Yang Liu, Liyun Zhang, Shanshi Deyi Hospital, Rheumatology, Taiyuan, China

Background: Rheumatoid arthritis (RA) is a highly disabling autoimmune disease, characterized by destruction of the cartilage and bone, which is difficult to reverse. Mesenchymal stem cell-derived exosomes (Mesenchymal stem cell-derived Exos) is an extra cellular vesicle, produced by MSCs in resting or stress state, which can simulate the tissue repair effect of its maternal cells and may be a new breakthrough point in the treatment of RA bone destruction.

Objectives: Study the effect of human umbilical cord mesenchymal stem cells derived exosomes (hUCMSC-exos) on bone destruction in Collagen induced arthritis (CIA) rats.

Methods: 1. After isolated by differential centrifugation, hUCMSCs was cultured in vitro, which was identified by morphology and surface markers. 2. Grouping: The CIA rats were randomly divided into CIA group, MTX group, hUCMSCs group, hUCMSC-exos low concentration group, hUCMSC-exos medium concentration group, hUCMSC-exos high concentration group, and a healthy control group. 3. Efficacy evaluation: the efficacy of hUCMSC-exos on CIA rats was evaluated by measuring joint swelling, arthritis index, micro-ct scanning and pathological score. The expression levels of RANKL and OPG in the serum of rats in each group were detected by RT-PCR technique, and the mRNA levels were detected by ELISA method, and the levels of the above factors in the synovial tissue of the ankle were detected by immunohistochemical staining technique, and the mRNA levels were detected by RT-PCR.

Results: 1. Effect evaluation: hUCMSC-exos can improve the arthritis index (AI) of CIA rats, and no definite side effect been found. In addition to hUCMSC-exos low concentration group, the joint synovial hyperplasia and inflammatory cell infiltration degree of each treatment group were significantly improved, comparing with that of CIA group. The Results of Micro-ct scanning suggested that the degree of osteoporosis in each intervention group was significantly improved comparing with that in the CIA group, and the improvement degree of hUCMSCs groups,MTX groups and hUCMSC-exos high concentration group was similar. 2. The detection of bone metabolism factor in Serum and synovial tissue: medium and high concentrations of hUCMSC-exos, hUCMSCs group and MTX group could down-regulate the level of RANKL in serum and synovial tissues of CIA rats and raise the level of OPG, and the effect of hUCMSC-exos in high concentration group was slightly obvious. Conclusion: hUCMSC-exos can improve bone destruction in CIA rats, and it is safe. hUCMSC-exos can mimic its mother cells and exert an inhibitory effect on bone destruction by regulating the imbalance of RANKL/OPG, and the effect is equal or better than its mother cells, and the high concentration group is superior to the low concentration group.

REFERENCES:

Disclosure of Interests: None declared

LOW ω-6:ω-3 RATIOS CAUSED REDUCTION IN CELLULAR INFILTRATION IN HUMAN SYNOVIOTES CELL LINE (SW922): GENE EXPRESSION STUDY

Abhay Harshulkar 1, Priya Kulkarni 2, Vanshika Srivastava 3, Ragini Vidyavati 4, Saurabh Yadav 4, Shreya Chitnnavis 5, Alankruta Senapaty 5, Bhavati Vidyapeeth University, Department of Cell and Molecular Biology, Pune, India; 2University of Tartu, Department of Pathophysiology, Tartu, Estonia; 3Bhavati Vidyapeeth University, Department of Pharmaceutical Biotechnology, Pune, India

Background: Alarming global increase in osteoarthritis (OA), coupled with off target existing palliative care poses nutriceuticals an extremely attractive alternative in this respect. Beyond meeting the basic nutritional demands, nutrition is thought to play a beneficial role in the management of chronic diseases. Interestingly, the off target effects of nutriceuticals are beneficial in reducing co-morbidities. Skewed ω-6: ω-3 fatty acids (FAs) in modern diets has been shown associated with increased number of inflammatory diseases including bone and cartilage metabolism. Considering this, due to their disease modifying actions, ω-3 FAs are thought to serve better in OA management.

Objectives: We here investigated the effect of different ω-6: ω-3 ratios on synovitis, a prominent OA pathology. The selected ratios are generally found in global diet.

Methods: Selected 6FAs - arachidonic acid (AA), eicosadienoic acid (EEDA), linoleic acid (LA) and gamma linoleic acid (DGLA) along with selected ω-3 FAs docosapentaenoic acid 5 (DPA) and α-linolenic acid (ALA) were tested on induced SW-982 cells, in common dietary ratios of 16:1, 8:1, 4:1, 2:1 and 1:1 for their effect on synovial inflammation. For induction, 50ng/ml tumor necrosis factor-α (TNF-α) was used for 72 hrs and the PUFAs were added in the culture media in the selected ratios (concentration of each PUF - 50μM). Finally, isolated cDNA was used to run quantitative RT-PCR for superoxide dismutase (SOD), interleukin-15 (IL-15), matrix metalloproteinase-1 (MMP-1) and vascular endothelial growth factor (VEGF).

Results: All the results LA:ALA of caused a remarkable reduction in MMP-1(P<0.001), Marginal reduction in VEGF was noted with 1:1, 4:1 and 8:1 ratios (P<0.005); 8:1 and 16:1 showed a significant increase (P<0.001). SOD did not show any particular trend but noteworthy increase was observed with 2:1, 8:1 and 16:1 ratios (P<0.001) while reduction with 1:1 and 4:1 ratios (P>0.001). IL-15 remained low for 1:1, 2:1and 4:1 while a marked up-regulation was noted for 8:1 and 16:1 ratios (P<0.001). In case of EDA:DPA, a consistent down-regulation of MMP-1was found with all the ratios excluding 4:1. The selected ratios were efficient against VEGF (P<0.001). A consistent high SOD was revealed by all the ratios of EDA:DPA, which remained comparable to control. Marginal reduction in IL-15 was noted with 1:1, 8:1 and 16:1 (P>0.05). Lastly, a significant MMP-1 reduction was caused by all the ratios of DGLA:DPA, except 2:1. Excluding 2:1, all the ratios were efficient against VEGF (P<0.001), increased levels of SOD remained comparable with TNF-α for 1:1 and 4:1; a trivial drop was noted with 2:1 and 8:1, followed by a noticeable reduction with 16:1. IL-15 showed a down-regulation with 1:1, 4:1, 8:1 ratios of DGLA: DPA.

Conclusion: Consequence of synovial inflammation in typical and atypical forms results in cartilage-loss, osteophytes and pain. FA ratios when used in equivalent, was found highly effective against IL-15, MMP1 and VEGF, whereas inflammation increases with increase in their proportions. Low SOD indicated lower oxidative-stress while it was up-regulated in response to high stress. FAs work in two distinct ways, membrane incorporation and metabolic modulation. Unsaturated fatty acid increase membrane fluidity and thus improves cell signaling. Analysis of treated and untreated cells showed improvement of FAs in cell membrane, which improves membrane fluidity and status of signal transduction.

Acknowledgement: This research work has been carried out as a part of OAIBGE2016-18, an international consortium supported by INNO-
Background: Beclin-1 is a component of the autophagy pathway necessary for formation of autophagosomes, contributing to autophagy-mediated cellular homeostasis. Enhancing autophagy through inhibition of mTOR activity, either via genetic deletion in chondrocytes or intra-articular injection of rapamycin, attenuates progression of surgically-induced models of osteoarthritis (OA). Retro-inverso TAT-Beclin-1 is a cell-permeable peptide which competes for binding to the endogenous Beclin-1 inhibitor GAPPR-1, thus promoting autophagy. It is unknown whether activation of Beclin-1 is sufficient to protect joints from osteoarthritis progression.

Objectives: For this study, we sought to determine if retro-inverso TAT-Beclin-1 could attenuate OA progression in a surgically-induced mouse model. Methods: Eight-week old C57BL/6 mice underwent destabilization of the medial meniscus (DMM) surgery to induce OA, or sham surgery as a control. Mice were injected intra-articularly with retro-inverso TAT-Beclin-1 (2 mg/kg in 5 μl) twice weekly for 2 or 9 weeks. Mice were sacrificed at 10-weeks post-surgery. Knee joints were stained with Safranin-O/Fast-green to evaluate collagen degeneration and Masson’s trichrome to determine degree of synovitis using OARSI scoring for mice. Sections were stained for α-SMA (myofibroblast) and CD45 (hematopoietic-origin cell) to evaluate changes in markers of fibrosis and inflammation, respectively.

Results: As opposed to the effects of mTOR deletion in cartilage or rapamycin treatment in joints, injection of retro-inverso TAT-Beclin-1 for 2 into knee joints of mice with DMM-induced OA had no effect on the degree of articular cartilage degeneration in the tibia or femur as compared to PBS-injected controls. However, in both sham and DMM mice, retro-inverso TAT-Beclin-1 for 2 or 9 weeks of treatment induced a pronounced thickening of the synovium with increased cell numbers and collagen deposition compared to PBS-treated mice. The increased number of synovial cells in 9-week treated mice did not show substantial expression of α-SMA or CD45+ cells, suggesting the increased number of cells and matrix in the synovium was independent of myofibroblast differentiation or inflammatory influx.

Conclusion: Contrary to our expected results, retro-inverso TAT-Beclin-1 did not attenuate cartilage degeneration. Rather, it promoted substantial synovial thickening that likely involved cell proliferation and collagen deposition. This severe fibrotic phenotype appears independent of myofibroblast differentiation or inflammation, normally associated with typical fibrotic responses. To evaluate the potential for dose responses with retro-inverso TAT-Beclin-1 in synovial joints, we are currently modifying our dosing strategy in an effort to determine possible disease modifying effects of this novel Beclin-1 activator.

Disclosure of Interests: None declared


FR00514

NEW DIAGNOSTIC BIOMARKER OF BONE TISSUE METABOLISM DISORDERS IN RHEUMATOID ARTHRITIS

I. Spewadowska1, J. Polakova2, B Zavodovsky3,2, Eugene Papichev2, Akhtryrendy Yury1,1, Treatment and Prevention of Joint Disease Laboratory/ Federal State Budgetary Institution «Zborovsky Research Institute of Clinical and Experimental Rheumatology», Volgograd, Russia Federation, 2Volgograd State Medical University, Volgograd, Russian Federation

Background: Bone mineral density and proteins/peptides determination in blood and urine as markers of bone resorption and formation are currently used to diagnose osteoporosis (OP) and metabolic bone diseases. However, these methods have some disadvantages for bone turnover evaluation. Recent evidence suggests that in RA changes in the secretion of hormones of white adipose tissue can be revealed [1,2,3,4]. One of them is Adiponectin possessing anti-inflammatory, anti-diabetic and anti-atherogenic properties. Changes in Adiponectin based cell death only in presence of TNF-α. Viability of SF was not affected by silencing of CBP.

Conclusion: Here we identified p300 as a major regulator of the proteasome in SF and provide first evidence for individual functions of CBP and p300 in regulating autophagy in SF.

Disclosure of Interests: Monika Wojciech: None declared, Marcel Gabathuler: None declared, Christoph Kolling: None declared, Matja Tomsic: None declared, Oliver Distler Grant/research support from: Prof. Distler received research funding from Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe to investigate potential treatments of scleroderma and its complications, Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with: Bayer, Boehringer Ingelheim, espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Serodapharm and UCB in the area of potential treatments of scleroderma and its complications. In addition, he had/has consultancy relationship within the last 3 years with: A. Menarini, Amgen, Abbve, GSK, Mepha, MSD, Pfizer and UCB in the field of arthritis and related disorders, Caroline Ospelt: None declared, Kerstin Klein: None declared.

Disclosure of Interests: None declared, I. Spewadowska declares being a co-author of a US and international patent (WO2016111654) for which licensed to Previation Inc., a startup company.


Disclosure of Interests: None declared, Christoph Kolling: None declared, Kerstin Klein: None declared.

Disclosure of Interests: None declared, Oliver Distler Grant/research support from: Prof. Distler received research funding from Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe to investigate potential treatments of scleroderma and its complications, Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with: Bayer, Boehringer Ingelheim, espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Serodapharm and UCB in the area of potential treatments of scleroderma and its complications. In addition, he had/has consultancy relationship within the last 3 years with: A. Menarini, Amgen, Abbve, GSK, Mepha, MSD, Pfizer and UCB in the field of arthritis and related disorders, Caroline Ospelt: None declared, Kerstin Klein: None declared.

Disclosure of Interests: None declared, I. Spewadowska declares being a co-author of a US and international patent (WO2016111654) for which licensed to Previation Inc., a startup company.


Disclosure of Interests: None declared, Christoph Kolling: None declared, Kerstin Klein: None declared.

Disclosure of Interests: None declared, Oliver Distler Grant/research support from: Prof. Distler received research funding from Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe to investigate potential treatments of scleroderma and its complications, Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with: Bayer, Boehringer Ingelheim, espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Serodapharm and UCB in the area of potential treatments of scleroderma and its complications. In addition, he had/has consultancy relationship within the last 3 years with: A. Menarini, Amgen, Abbve, GSK, Mepha, MSD, Pfizer and UCB in the field of arthritis and related disorders, Caroline Ospelt: None declared, Kerstin Klein: None declared.

Disclosure of Interests: None declared, I. Spewadowska declares being a co-author of a US and international patent (WO2016111654) for which licensed to Previation Inc., a startup company.