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beneficial in the treatment of degenerative musculoskeletal problems. The purpose of this study is to evaluate PRP treatment efficacy on degenerated cartilage.
Objectives: In this study, we aimed to determine the efficacy of platelet-rich plasma (PRP) on mechanically damaged chondrocyte cells by using different dose, different duration of exposure and different methods of activation of platelet.
Methods: Human source chondrocytes (CHON-001 ATTC CRL-2846) were used in thestudy. Chondrocyte cells were produced in appropriate medium and an experimental cartilage model was created. Theplatelet-rich plasma was produced from platelets obtained by apheresis in the laboratory, from blood of volunteer. Theplatelet-rich plasma was adjusted at five different doses as $4{,}8x10^6$, $2{,}4x10^6$, $1.2x10^6$, $6x10^5$, $3x10^5$. The first group of platelet rich plasma was left intact, the second group was detonated within seven minutes by applying ultrasound waves in water, the third group was activated with calcium chloride and thefourth group was determined as control group. Using a ten microliter pipette tip, a linear damage to theopposite side was created at the widest part of the well. Cell migration was monitored at 0-4-8-24 and 48 hours at x10 magnification by in vitro microscopy and wound healing was evaluated by photographing. Migration intervals were determined quantitatively using the program named Image J.
Results: When the rates of recovery were compared to the groups, no significant improvement was observed in the intact and detonated platelet groups at 4-8 and 24 hours compared to the control group. In the third group which was activated with calcium, no significant improvement was observed in all doses at 4 and 8 hours compared to the control group. However, at the 48th hours there was a significant improvement in the doses of $1.2x10^6$, $2.4x10^6$and $4.8x10^6$compared to the control group ($p<0.0001$).
There were significant differences in intact and detonated platelets at $3x10^5$and $6x10^5$doses at 48th hours compared to controlgroup ($p<0.0001$). Significant improvement was observed in all groups at levels of $1.2x10^6$and a bove ($p<0.0001$).
When evaluated in terms of activation, there was a significant improvement in the exploded and intact groups at the 48th hour, compared to the calcium-activated group at doses of $3x10^5$ and $6x10^5$ ($p<0.01$).
Conclusion: Cartilage damage is the main pathology in the pathogenesis of osteoarthritis. All doses of PRP used in the study contributed to improvement. Meanwhile, the most critical parameter for platelet migration was timing and significant improvement was started after 48 hours.

REFERENCES

[1] Nguyen RT, Borg-Stein J, McInnis K. Applications of platelet-rich plasma in musculoskeletal and sports medicine: an evidence-based approach. PM R 2011;3(3):226-50.
[2] Filardo G, Kon E, DiMatteo B, Merli ML, Cenacchi A, Fornasari PM, et al. Platelet-rich plasma vs hyaluronicacid to treat knee degenerative pathology: study designand preliminary results of a randomizedcontrolledtrial. BMC MusculoskeletDisord2012;13:229.
[3] Spaková T, Rosocha J, Lacko M, Harvanova D, Gharaibeh A. Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid. Am J Phys Med Rehabil 2012;91(5):411–7.
[4] Virchenko O, Aspenberg P. How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay between early regeneration and mechanical stimulation. Acta Orthop 2006;77(5):806-12.

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AB0106 CHANGES IN THE MIRNA PROFILE AND HYPOXIC BEHAVIOUR OF HUMAN CHONDROCYTES BY THERAPEUTIC NUCLEAR MAGNETIC RESONANCE THERAPY (NMRT)

Birgit Lohberger[1], Lukas Weigl[2], Nicole Stuendl[1], Anda Mann[2], Andreas Leithner[1], Werner Kullich[3], Bibiane Steinecker-Frohnwieser[4]. [1]*Medical University of Graz, Department of Orthopaedics and Trauma, Graz, Austria*; [2]*Medical University of Vienna, Division of Special Anaesthesia and Pain Medicine, Vienna, Austria*; [3]*Ludwig Boltzmann Institute for Arthritis and Rehabilitation, Saalfelden, Austria*; [4]*Ludwig Boltzmann Institute for Arthritis and Rehabilitation, Groebming, Austria*

Background: Therapeutically applied nuclear magnetic resonance (NMRT) is discussed to participate in repair processes regarding cartilage and influences pain signaling. Studies concerning NMR therapy implemented within the treatment of patients with degenerative rheumatic diseases outlined pain reduction as the main clinical outcome (1). NMRT is also known to lead to improvements in pain from patients with knee OA due to a chondroprotective effect on the articular cartilage. In spite of this significant reduction in pain, the mechanism of action of NMRT at the cellular level remains to be elucidated.
Objectives: To substantiate the application of NMRT the aim of this work targets the underlying mechanisms at the cellular level. We investigated NMRT induced changes of the miRNA profile of human healthy and OA chondrocytes and studied the respective miRNA targets. Based on the fact that articular cartilage functions as an avascular and hypoxic connective tissue we were further able to demonstrate that NMRT modulation seems to be more pronounced under hypoxic conditions.
Methods: Human primary chondrocytes and the chondrocyte cell line Tc28/2a were used for the experiments.
RNA was extracted using RNEasy Mini Kit and was used as input for the Thermo Fisher Ion Total RNA-Seq Kit v2. Sequencing was performed on Ion Proton sequencer using the Ion PI Hi-Q™ Sequencing 200 system. Signal processing and base calling was performed using Torrent Suite version 5.6. Hypoxic conditions were established and enabled cell growth in presence of 1-5% O2. Expression of miRNAs and target proteins was studied by a standard PCR procedure as well as protein detection by western blot. HDAC activity was measured by HDAC-Glo I/II assay.
Results: Characterization of the miRNA profile showed a slight up regulation of miR-24-1-5p and miR-502-5p while miR-25-5p and miR-365a-5p was down regulated. For miR-365a-5p known to directly targeting HDAC and NFkB a decrease of HDAC activity by NMRT was detected. The miR-25-5p target COX2 was changed in expression by NMRT whereas no influence on CDK4 knowing to be controlled by miR-24-1-5p was detected. NMRT treatment of chondrocytes under hypoxic conditions (0-5 % O_2) changed the expression profile with respect to NOS, IGF2, PDGF and IGFBP and a change in the expression of Hif1/2 under the influence of IL1b was observed. The hypoxic conditions changed apoptotic behavior of the cells, NMRT showed no influence.
Conclusion: A closer look into the mechanism of the NMRT at the cellular level revealed a modulatory effect on miRNA, their regulatory units and chondrocytes under hypoxic conditions. The results underline our former results indicating that NMRT counteracts IL-1b induced changes which mean that pain reduction by NMRT might be due to NMRT holding against inflammatory mechanisms under OA.

REFERENCES

[1] Kullich W, Overbeck J, Spiegel HU; Journal of Back and Musculoskeletal Rehabilitation 2013, 26:93-104.
[2] Steinecker-Frohnwieser B, Kullich W, Mann A, Kress H, Weigl L; Exp. Clin. Rheum. 2018, 36, 2:0294-0301.

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AB0107 S-ALLYL-L-CYSTEINE ATTENUATES INFLAMMATION RELATED OXIDATIVE STRESS PARAMETERS AND INCREASES ADHESION CAPACITY OF PRIMARY HUMAN OSTEOARTHRITIC ARTICULAR CHONDROCYTES

Zubeyir Elmazoglu[1], Zehra Aydin Bek[1], Berna Göker[2], Berivan Bitik[3], Cem Nuri Aktekin[4], Mehmet Engin Tezcan[5], Cimen Karasu[1]. [1]*Gazi University Faculty of Medicine, Cellular Stress Response and Signal Transduction Research Laboratory, Department of Medical Pharmacology, Ankara, Turkey*; [2]*Gazi University Faculty of Medicine, Rheumatology, Ankara, Turkey*; [3]*Baskent University, Rheumatology, Ankara, Turkey*; [4]*Yıldırım Beyazıd University, Orthopedics andTraumatology, Ankara, Turkey*; [5]*Kartal Dr. Lutfi Kirdar Training and Research Hospital, Rheumatology, Istanbul, Turkey*

Background: Osteoarthritis (OA) is characterized by progressive destruction of the articular cartilage, and chondrocytes, the only cells in articular cartilage, are in charge of maintaining the homeostasis of articular cartilage via modulating extracellular matrix anabolism and catabolism [1]. Due to the association of the degree of oxidative degeneration of chondrocytes and OA, preventing impaired redox signalling and oxidative death of chondrocyte are suggested as potential targets to relieve OA[2]. In this respect, some photochemicals have been shown to be potential agents for preventing or treating OA due to their antioxidant and anti-inflammatory properties [3].
Objectives: We studied the effects of an antioxidant S-allyl-cysteine (SAC), a major sulphur-containing amino acid compound of garlic[4], on the redox system, and its associations with the proliferation rate and index of human OA chondrocytes(OACs).
Methods: Chondrocytes were isolated from the joint cartilages of OA patients (grade 4, meanage= 66 years, BMI= 29.7 ± 4.4 kg/m^2).The alterations in cell proliferation (MTT), adhesion profile (RTCA-iCELLigence System), reactive oxygen species generation (ROS), lipid hydroperoxide levels (LPO), HNE-protein adduct levels (HNE), AGE-protein adduct levels(AGE), 3-nitrotyrosine levels (3-NT),

glutathione peroxidase activity (GPx), and inflammatory progenitors ICE/caspase-1 (ELISA) were determined.

Results: SAC increased OAC's proliferation rate and adhesion profile at relatively low concentrations (1, 10 and 100 nM),but inhibited at higher concentrations (1-100 μM). SAC (1 nM-10 μM) inhibited ROS, LPO and 3-NT, but not HNE- and AGE-modified proteins levels. SAC increased GPx but drastically down regulated ICE/caspase-1, indicating a potential redox regulating and anti-inflammatory effect.

Conclusion: Results suggest that SAC has favourable effects on OA chondrocytes through protecting proliferation capacity and ameliorating redox-mediated inflammatory pathways. Further studies are needed to investigate its therapeutic potential in patients with OA.

REFERENCES

[1] Lepetsos P, Papavassiliou AG. ROS/oxidative stress signalling in osteoarthritis. Biochim Biophys Acta. 2016 Apr;1862(4):576-591.

[2] Drevet S, et al. Reactive oxygen species and NADPH oxidase 4 involvement in osteoarthritis. Exp Gerontol. 2018 Oct 1;111:107-117.

[3] Goker B, et al. Luteolin Modulates Glyco-Lipo-Oxidative Protein Modifications and Inhibits Inflammatory Cytokine Release in Human Osteoarthritic Articular Chondrocytes: Comparison with Colchicine. 2018 ACR/ARHP Annual Meeting. Abstract Number: 1990.

[4] QianYQ, et al. Down regulating PI3K/Akt/NF-κB signalling with allicin for ameliorating the progression of osteoarthritis: in vitro and vivo studies. Food Funct. 2018 Sep 19;9(9):4865-4875.

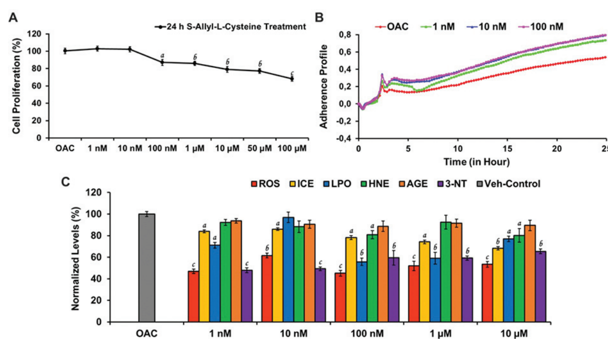

Figure.. Cell proliferation of human primary chondrocytes (A) and dynamic monitoring of adhesion profile of human primary chondrocytes during SAC (B) treated with SAC (24 h). ICE/caspase-1, ROS, LPO, HNE, AGE, 3-NT levels after SAC (24 h) treatment (C). ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 vs OAC. (Bland-Altman Statistics)

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AB0108

THE ROLE OF CD70 IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

Su-Jin Yoo, Seong Wook Kang, Jinhyun Kim, In Seol Yoo, Chan Keol Park, Ha-Reum Lee. *Chungnam National University Hospital, Rheumatology division, Dept. of Int. Med., Daejeon, Korea, Rep. of (South Korea)*

Background: Rheumatoid arthritis (RA) is characterized by inflammation and cellular proliferation in the synovium. Activated lymphocytes and proinflammatory molecules are important in the pathogenesis of RA.

CD70 belongs to the tumor necrosis factor (TNF) ligand superfamily and is typically present on activated B and T lymphocytes, natural killer cells and mature dendritic cells.

CD70 expressing $CD4^{+}$ T cells are enriched in the peripheral blood and synovial fluid of patients with RA and promote autoimmunity via co-stimulatory CD70-CD27 interaction.

CD70 expression is associated with aggressive phenotype of cancer cells and it is mediated by hypoxia inducible factor 2α (HIF-2α).

Objectives: In this study, we examined the presence of CD70 on the surface of fibroblast-like synoviocyte (FLS) of patients with RA (RA-FLS) and investigate the role of CD70 in the pathogenesis of RA associated with HIF-2α.

Methods: RA FLS were obtained from 7 patients with RA who were undergone operation like total knee replacement or synovectomy. All patients were fulfilled the 2010 ACR-EULAR classification criteria for RA.

CD70 and HIF-2α messenger ribonucleic acid (mRNA) were analyzed in RA-FLS by *quantitative polymerase chain reaction* (*qPCR*).

CD70 and CD27 on the surface of RA-FLS were stained by PE-Anti CD70 antibody and PerCP-Cy5.5-CD27 antibody respectively and evaluated by flow cytometry.

Same experiments were performed after treatment with interleukin (IL)-17, TNF-α and HIF-2α blocking antibody (Anti HIF-2α antibody).

Results: CD70 and HIF-2α mRNA in the RA-FLS were elevated after treatment with IL-17 and TNF-α (Figure 1, 2).

The level of CD70 expression on the surface of RA-FLS was elevated after stimulation with IL-17 and TNF-α (Figure 3). And it was lowered after treatment with HIF-2α blocking antibody as dose dependent pattern (Figure 3).

CD27 wasn't present on the surface of RA-FLS (Figure 4).

Conclusion: We identified the expression of CD70 on the surface of RA-FLS.

And in inflammatory conditions like stimuation with IL-17 and TNF-α, both CD70 and HIF-2α mRNA were increased. The level of CD70 on the surface of RA-FLS also elevated by treatment with IL-17 and TNF-α.

The result of decreased level of CD70 after treatment with anti HIF-2α antibody suggest that CD70 expression on the surface of RA-FLS is associated with HIF-2α.

From these results, we expect that CD70-targeted therapy associated with HIF-2α may be effective for treatment with RA.

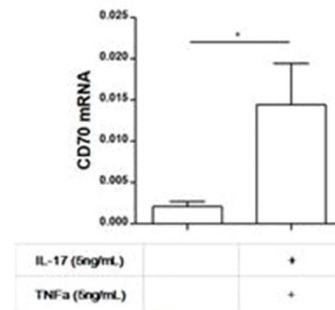

Fig1. The result of CD70 mRNA after treatment with IL-17 & TNFa (5 RA FLS, * P = 0.008)

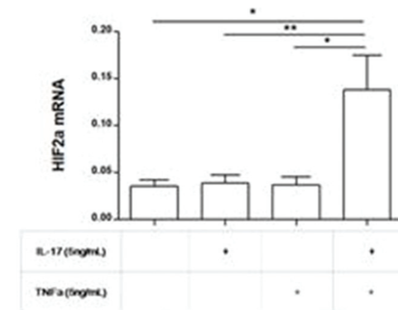

Fig2. The result of HIF2a mRNA after treatment with IL-17 & TNFa (7 RA FLS, * P = 0.019, ** P = 0.039)

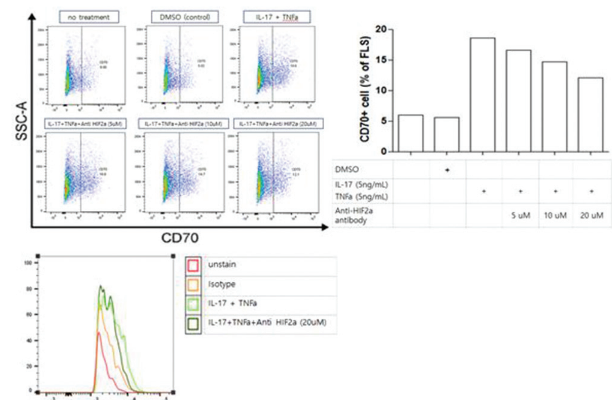

Fig3. The result of CD70 on the RA FLS after treatment with IL-17, TNFa and anti-HIF2a antibody by flow-cytometry

REFERENCES

[1] Han, B. K., Olsen, N. J., & Bottaro, A. (2016). The CD27-CD70 pathway and pathogenesis of autoimmune disease. Semin Arthritis Rheum, 45(4), 496-501.

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