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 ATCC CRL-2846) were used in the study. Chondrocyte cells were produced in appropriate medium and an experimental cartilage model was created. The platelet-rich plasma was produced from platelets obtained by apheresis in the laboratory, from blood of volunteer. The platelet-rich plasma was adjusted at five different doses as 4.8×10⁵, 2.4×10⁵, 1.2×10⁵, 6×10⁴, 3×10⁴. 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 the fourth group was determined as control group. Using a ten microliter pipette tip, a linear damage to the chondrocyte 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 1.2×10⁵, 2.4×10⁵and 4.8×10⁵compared to the control group (p <0.0001).

There were significant differences in intact and detonated platelets at 3×10⁵and 6×10⁵doses at 48th hours compared to control group (p <0.0001). Significant improvement was observed in all groups at levels of 1.2×10⁵and a b o v e (p <0.0001).

When evaluated in terms of activation, there was a significant improvement in the intact and detonated groups at 48th hours, compared to the calcium-activated group at doses of 3×10⁵and 6×10⁵(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


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AB0106

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

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Background: Therapeutically applied nuclear magnetic resonance (NMRT) is discussed to participate in repair processes regarding cartilage and influences pain signaling. Studies concerning NMRT 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 healthy human and OA chondrocytes and studied the respective miRNA targets. Based on the fact that articular cartilage functions as an avascular and anoxic 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 II/I 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 % O2) changed the expression profile with respect to NOC, IGF2, PDGF and IGBP and a change in the expression of Hif1β under the influence of IL1b was observed. The hypoxic conditions changed apotptic 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 interactions IL-1b induced changes which mean that pain reduction by NMRT might be due to NMRT holding inflammatory mechanisms under OA.

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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

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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 signaling 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 (OCs).

Methods: Chondrocytes were isolated from the joint cartilages of OA patients (grade 4, meanage= 66 years, BMI= 29.7 ± 4.4 kg/m²). 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).