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THU0021 CLINICAL SIGNIFICANCE OF WEIGHT LOSS ON THE LEVEL OF PROINFLAMMATORY CYTOKINES IN PATIENTS WITH OSTEOARTHRITIS

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Background: Obesity is a condition that prolongs chronic inflammation and promotes synthesis and secretion of pro-inflammatory factors by adipose tissue, such as classical cytokines, tumor necrosis factor- α (TNF- α), adipokines (leptin, adiponectin, resistin) and other newly identified proinflammatory factors (hemerin, lipokain, serum amyloid protein 3) [1,2,3,4,5]. Nowadays one of the most actively studied adipokines is nicotinamide phosphoribosyltransferase (visfatin, Nampt).

Objectives: We investigated the relationship the effect of weight loss over 5 kg on the clinical manifestations of OA and Nampt serum levels in patients with OA.

Methods: We observed 160 patients with different forms of OA ranged in age from 36 to 78 years, of whom there were 104 (65%) women (mean age 52.08 $\pm$ 1.58 years), and 56 (35%) of men (mean age - 54.07 $\pm$ 2.0 years) and the control group (60 healthy persons) with no complaints of pain in the joints over a lifetime, and without clinical signs of OA. Nampt level in serum was determined by ELISA using a commercial test systems.

Results: As overweight patients were recruited in the study, hypocaloric diet low in animal fats and physiotherapy has been recommended to all participants. The positive dynamics in body weight loss over 5kg within 3 months has been achieved by 36 patients (23%). All patients were divided into two groups to study the effect of weight loss on the clinical manifestations of OA. These data proves that obesity may be an important risk factor for OA progression. As a result, weight loss results in decreasing metabolic disorder severity. In the second group of patients we have seen a decrease in all the parameters, but a significant difference has been observed only in the level of CRP, level of pain at rest and during walking according to VAS scale and total index on the WOMAC. However, patients with weight loss over 5 kg had significantly greater positive dynamics of clinical parameters than in the second group without weight loss. This fact is probably explained by the decreased activity of inflammatory process after OA therapy and weight reduction.

Conclusion: Thus, as a result of our study patients with OA with weight loss of more than 5 kg had more obvious pain relief than patients with the original weight. These findings suggest that there is a possible role of visfatin in the pathogenesis of osteoarthritis. All patients with OA with a BMI over 25 kg/m² are recommended to lower their weight to decrease the mechanical stress on the joints, and also to reduce the severity of inflammation and metabolic disorders.

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THU0022 ETANERCEPT INCREASES AUTOPHAGY AND REDUCES APOPTOSIS INDUCED BY TNF-ALPHA EXPOSURE IN ENDOTHELIAL CELLS IN VITRO

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Background: Rheumatoid arthritis (RA) is associated with high incidence of cardiovascular events, mainly due to accelerated atherosclerosis and endothelial dysfunction (1). Apoptosis in endothelial cells has been associated with vascular wall damage, contributing to atherosclerotic plaque formation (2). At the contrary, autophagy may play a protective role in preventing development of atherosclerosis. However, the exact mechanism which controls the autophagy of endothelial cells is still unknown. Tumor necrosis factor alpha (TNF- α) is one of the leading cytokines involved in the pathogenesis of RA. Several evidences suggest a pro-atherogenic effect of TNF- α in patients with RA. Etanercept (ETA) and other TNF- α inhibitors are effective treatments for joint inflammation in RA, but their effect on endothelial dysfunction is still not well understood.

Objectives: The aim of this study was to investigate *in vitro*, the effects of ETA treatment on endothelial cells exposed to TNF- α , with particular attention on apoptosis levels and autophagy pathway.

Methods: *In vitro* effects of ETA and TNF- α on endothelium were evaluated using human umbilical vein cell line EA.hy926. Cells were treated with ETA (15 μ g), TNF- α (10 ng/ml), alone or in combination. An untreated control cell culture was also performed. After 24 hours, apoptosis levels and autophagy have been evaluated. Apoptosis was analyzed by flow cytometry using a FITC-conjugated annexin V and propidium iodide apoptosis detection kit (3); autophagy was analyzed by western blot for the expression level of the autophagic markers LC3-II and p-62 (4).

Results: TNF- α treatment significantly increased apoptosis in endothelial cells compared both to untreated and ETA-treated cells ($p=0.0067$ and $p=0.00187$ respectively) [Figure 1]. The co-treatment with TNF- α and ETA significantly reverted the increase of apoptosis levels ($p=0.001$ versus TNF α alone). Both ETA and TNF α treatments significantly increased the expression level of LC3II in treated cells when compared to untreated cells ($p=.18$ and $p=.3$ respectively). The co-treatment induced an additive effect on autophagy levels ($p<0.0001$ versus untreated cells). At last, the expression level of p-62 showed an inversely proportional trend to LC3II levels [ETA ($p=0.0001$), TNF- α $p=.1$), ETA+TNF- α $p=.5$) versus untreated cells, respectively] [Figure 2].

Conclusion: Our results show that treatment with Etanercept have a protective effect on endothelial cells *in vitro*, reversing the apoptosis induced by TNF- α . Moreover, this is the first study that supports a possible effect of Etanercept in increasing autophagy level in endothelial cells. Reducing TNF α -induced apoptosis, Etanercept treatment may lead to an improvement of endothelial function and to a reduction of accelerated atherosclerosis.

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Abstract THU0022 Figure 1.

Abstract THU0022 Figure 1.

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THU0023 PARADOXICAL EFFECT OF INTERLEUKIN 1 BETA CYTOKINE ON COLLAGEN TYPE I SYNTHESIS IN OSTEOBLAST-LIKE CELLS

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Background: Osteoblasts are the bone forming cells that responsible for the synthesis of collagen type I and mineralization of bone during initial bone formation and later bone remodeling. Abnormalities in osteoblasts phenotype and activity occur in common bone diseases including osteoarthritis (OA). Studies showed that osteoarthritic osteoblasts secrete a collagen type I homotrimer of $\alpha 1$ chains, which is phenotypically distinct from the normal heterotrimer formed by two $\alpha 1$ chains and one $\alpha 2$ chain. Cytokines released during OA initiation or progression may interfere with osteoblastic function in the bone matrix. Interleukin 1 beta (IL-1 β) is one of the major inflammatory cytokines implicated in the pathogenesis of OA; however, the biological response of osteoblasts to the cytokine is only partly understood.

Objectives: Our aims were to clarify the effects of IL-1 β on collagen type I synthesis in osteoblast-like cells and to explore further possible relationship between collagen type I synthesis and mineralization.

Methods: At confluence, MG63 osteoblast-like cells in osteogenic media were stimulated with low (1 ng/ml) or high (10 ng/ml) dose of recombinant human IL-1 β at several incubation times; 1, 2, 3, 4 and 5 hours. Non-stimulated MG63 cells were grown as control at indicated time points. Cell viability was tested by using the PrestoBlue reagent. Total collagen production was evaluated by Picro-sirius red precipitation method. Secretion of homotrimer collagen type I alpha 1 (COL1A1) and mineralization at exposure time of 1-, 3- and 5- hour were determined by immunofluorescence staining of COL1A1 antibody and Alizarin Red S staining, respectively.

Results: IL-1 β showed no statistical evidence in influencing cell viability at the time and dose tested compared to control. We found that IL-1 β significantly ($p < 0.05$) increased collagen content at short exposure time (1-hour), while significantly ($p < 0.05$) decreased collagen content at longer exposure time (5-hour), in a dose-dependent manner. Immunofluorescence staining showed increased of homotrimer COL1A1 at 1-hour exposure and decreased of homotrimer COL1A1 at 5-hour exposure of IL-1 β in the MG63 cells. IL-1 β stimulated the formation of mineralized nodules at all exposure time.

Conclusion: We demonstrated for the first time of the paradoxical effect of IL-1 β on collagen type I synthesis in osteoblast-like cells. Increased mineralization in low and high homotrimer type I collagen condition may possibly explain the abnormal mineralization in osteoarthritic bone.

Paradoxical role of IL-1 β in osteoblast may generate a different signal that regulates osteoblast markers expressed in the heterogenous subchondral bone changes in OA. Understanding these mechanisms could pave the way towards targeted therapeutic interventions.

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THU0024 INHIBITION OF CELL PROLIFERATION AND PROMOTION OF INTERLEUKIN-8 PRODUCTION BY T-614 IN CULTURED HUMAN AORTIC ADVENTITIAL FIBROBLASTS

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Background: Takayasu's arteritis (TA) is an inflammatory fibrosing arteritis that affects predominantly the aorta and its main branches. Recently, growing evidence supports that adventitial fibroblasts play an essential role in vascular inflammation [1]. Tumor necrosis factor- α (TNF- α) can reportedly induce inflammation of vascular adventitial fibroblasts [2]. T-614 (iguratimod), a novel disease-modifying antirheumatic drug, has been widely used in rheumatoid arthritis in China and Japan [3]. However, the effect and mechanism of T-614 on TA have received little attention.

Objectives: We report the effects of T-614 on cell proliferation and interleukin-8 (IL-8) production in cultured human aortic adventitial fibroblasts (HAAF), and explored its possible effect on the treatment of TA.

Methods: HAAF were cultured with 0, 5, 50, 100, or 250 μ g/ml T-614 in the absence or presence of 10 ng/ml TNF- α in vitro. Cell viability of HAAF was determined by a modified MTT assay. Supernatant IL-8 level was measured by enzyme linked immunosorbent assay.

Results: (1) After subculture, HAAF were polygonal or spindle-shaped under the microscope (Figure 1A, B). (2) In the presence of TNF- α , compared with the contrast group, cell viability of HAAF significantly decreased in 50, 100, and 250 μ g/ml T-614 treatment groups (OD value: $P < 0.01$, $P < 0.001$, $P < 0.001$, respectively; survival fraction (SF): $P < 0.05$, $P < 0.001$, $P < 0.001$, respectively) (Table I, Figure 2). However, there was no significant difference in cell viability between TNF- α stimulated and unstimulated groups at the same concentration of T-614. In the absence and presence of TNF- α , T-614 suppressed HAAF cell viability dose-dependently (OD value: $r = -0.915$, $P = 0.000$ and $r = -0.926$, $P = 0.000$, respectively; SF: $r = -0.897$, $P = 0.000$; $r = -0.885$, $P = 0.000$, respectively). (3) In the absence of TNF- α , compared with the contrast group, IL-8 level in 5 and 100 μ g/ml T-614 treated groups were significantly higher ($P < 0.05$); in the presence of TNF- α , IL-8 level in 5, 50, and 100 μ g/ml T-614 treated groups were significantly higher ($P < 0.001$, $P < 0.001$, $P < 0.01$, respectively) (Figure 3). There was a negative correlation between supernatant IL-8 level and the concentration of T-614 in groups stimulated with TNF- α ($r = -0.670$, $P = 0.000$). TNF- α increased IL-8 level in the control group and various concentrations of T-614 treated groups (all $P < 0.001$).

Table I. Effects of T-614 on TNF- α stimulated cell viability of HAAF (OD value)

T-614 (μ g/ml)	OD value	
	0 ng/ml TNF- α	10 ng/ml TNF- α
0	0.424 $\pm$ 0.038	0.414 $\pm$ 0.042
5	0.404 $\pm$ 0.050	0.440 $\pm$ 0.054
50	0.347 $\pm$ 0.061***	0.358 $\pm$ 0.040**
100	0.267 $\pm$ 0.034***	0.302 $\pm$ 0.039***
250	0.096 $\pm$ 0.019***	0.092 $\pm$ 0.009***

TNF- α , tumor necrosis factor- α ; HAAF, human aortic adventitial fibroblasts. Data are shown as mean $\pm$ SD of 3 independent experiments, each in triplicate. *** $P < 0.001$, ** $P < 0.01$ vs. control group (0 μ g/ml T-614) within each group alone.

Conclusion: T-614 can inhibit the proliferation of HAAF and promote IL-8 production; therefore, it may provide a new immunotherapeutic intervention for TA.

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