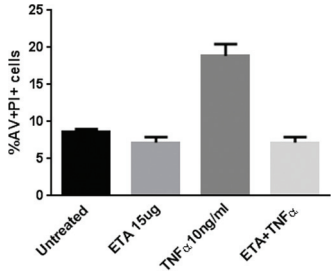
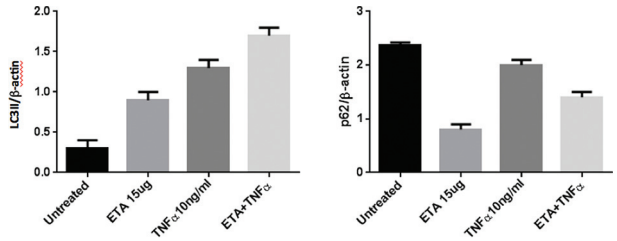


Abstract THU0022 Figure 1.



Abstract THU0022 Figure 1.

Disclosure of Interests: None declared
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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 α1 chains, which is phenotypically distinct from the normal heterotrimer formed by two α1 chains and one α2 chain.
Cytokines released during OA initiation or progression may interfere with osteoblastic function in the bone matrix. Interleukin 1 beta (IL-1β) 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-1β 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-1β 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-1β showed no statistical evidence in influencing cell viability at the time and dose tested compared to control. We found that IL-1β 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-1β in the MG63 cells. IL-1β stimulated the formation of mineralized nodules at all exposure time.
Conclusion: We demonstrated for the first time of the paradoxical effect of IL-1β 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-1β 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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Disclosure of Interests: None declared
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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 μg/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 μg/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 μg/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 μg/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(μg/ml)	OD value	
	0 ng/ml TNF-α	10 ng/ml TNF-α
0	0.424±0.038	0.414±0.042
5	0.404±0.050	0.440±0.054
50	0.347±0.061***	0.358±0.040**
100	0.267±0.034***	0.302±0.039***
250	0.096±0.019***	0.092±0.009***

TNF-α, tumor necrosis factor-α; HAAF, human aortic adventitial fibroblasts. Data are shown as mean ± SD of 3 independent experiments, each in triplicate. $^{***}P < 0.001$, $^{**}P < 0.01$ vs. control group(0μg/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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