OBJECTIVES: To investigate the effect and mechanism of T-614 on TA. Methods: HAAF were cultured with 0, 5, 50, 100, or 250 μg/ml T-614 and 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, HAAF significantly decreased in 50, 100, and 250 μg/ml T-614 treatment groups (OD value: P<0.01, P<0.001, respectively; survival fraction (SF): 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, IL-8 production was significantly higher (P<0.05); in the absence of TNF-α, IL-8 level in 5 and 100 μg/ml T-614 treated groups were significantly higher (P<0.01). 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<0.01). TNF-α increased IL-8 level in the control group and various concentrations of T-614 treated groups (all P<0.001).


Abstract THU0024 Figure 1


Abstract THU0024 Figure 2

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The Janus kinase inhibitors (JAKi) peficitinib and filgotinib are currently examined in clinical trials for treatment of rheumatoid arthritis. Both inhibitors are well tolerated up to doses causing Cmax values higher than 1 μM [1,2]. This is in contrast to the approved dosages of tofacitinib and baricitinib reaching Cmax values below 0.5 μM [3,4]. However, it is not known if the higher concentrations of peficitinib or filgotinib offer a benefit in treatment of rheumatoid arthritis.

Objectives: The aim of the study compared the effect of different JAKi on inflammatory response and functional behavior of fibroblast-like synoviocytes from patients with RA (RASF).

Methods: Human RASF were isolated and pretreated with JAKi. After stimulation with IL-1β and JAKi with/without soluble IL-6 receptor (sIL-6R) the levels of IL-6 and MMP-3 were measured in supernatants by ELISA. The effect of different JAKi on proliferation of RASF was determined by a BrdU-incorporation assay. The influence of peficitinib on migration of RASF towards a FCS gradient was examined. For short-term adhesion assays, cells were treated with JAKi, detached and seeded in culture plates. The plates were extensively shaken and adherent RASF quantified by counting crystal violet stained cells. Cell viability, cytotoxicity and apoptosis were measured using commercially available assays.

Results: The IL-1β (10 ng/ml) dependant IL-6 release of RASF was decreased by peficitinib (62%, p<0.001) and by filgotinib (30%, p<0.05, n=7) at 5 μM. Peficitinib also decreased the IL-6 release at 1 μM (24%, n=7). In contrast to filgotinib, the JAK-inhibition with peficitinib reduced synovial fibroblasts (SF) are known key effector cells of cartilage destruction in inflammatory arthritides such as RA, tenocytes are a major component of tendons and entheses and play a central role in tendon inflammation observed in PsA.

Objectives: To investigate whether PsASF and tenocytes show significant interactions while being stimulated with the above cytokines alone as well as in combination with the aim to find out whether these may contribute to the pathogenesis of PsA.

Methods: SF were isolated from patients with PsA undergoing joint surgery. Human tenocytes were acquired commercially and isolated from hamstring tendon tissue of patients undergoing hamstring tendon ACL reconstruction. PsASF and tenocytes were stimulated with IL-1β, TNF-α, IFN-γ, IL-15 and IL-23 alone and in combination. Direct cell co-culture experiments were performed at a 1:1 ratio of both cell types in parallel to experiments with single cell type cultures. IL-6 levels were measured by ELISA to quantify the immunological activation of the cells.

Results: PsASF as well as tenocytes showed strong responses to IL-1β (tenocytes [173-fold, n=3]; PsASF [56-fold, n=3]) and TNF-α (tenocytes [10-fold, n=3]; PsASF [9-fold, n=3]) stimulation regarding IL-6 secretion. IFN-γ alone had only minimal effects on both cell types but acted synergistically when applied together with IL-1β (tenocytes [218-fold, n=3]; PsASF [112-fold, n=3]) and TNF-α (tenocytes [24-fold, n=3]; PsASF [119-fold, n=3]). IL-15 and IL-23 alone showed no effect but the data suggest a small antagonistic effect against IL-1β (tenocytes IL-15 [21%], IL-23 [27%], n=3; PsASF IL-23 [19%], n=3) and TNF-α induced IL-6 secretion. Overall, PsASF and tenocytes showed similar responses in the single cell type stimulation experiments. Co-culturing PsASF and tenocytes did not reveal any synergistic or antagonistic interactions in regards to any of the cytokines used.

Conclusion: Our data suggest that tenocytes and PsASF do not interact in a way that would promote inflammation within the synovio-enthesal complex. Also, as far as the induction of IL-6 is concerned, PsASF and tenocytes are not major target cells of IL-15 and IL-23. IFN-γ, however, may be able to promote inflammation in combination with other cytokines in both cell types.

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THU0025

Abstract THU0025 Figure 2

THU0026

TARGETING SYNOVIAL FIBROBLASTS IN RHEUMATOID ARTHRITIS BY PEFICITINIB AND FILGOTINIB

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Background: The Janus kinase inhibitors (JAKi) peficitinib and filgotinib are currently examined in clinical trials for treatment of rheumatoid arthritis. Both inhibitors are well tolerated up to doses causing Cmax values below 0.5 μM [3,4]. How-