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SAT0056

TAS5315, A NOVEL BRUTON'S TYROSINE KINASE INHIBITOR, DEMONSTRATES POTENT EFFICACY AGAINST BONE DESTRUCTION IN AN ANIMAL MODEL FOR RHEUMATOID ARTHRITIS

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Background: Bone erosions and cartilage damages are a pathological hallmark of rheumatoid arthritis (RA) and are associated with poor functional outcome¹. Aberrant activations of osteoclasts induced by receptor activator of nuclear factor κ B ligand (RANKL)² are involved in the bone erosions of RA. It has also been recently shown that under chronic inflammatory conditions such as RA, inflammatory cytokines in joints induce pathological osteoclast differentiation and cause excessive bone resorption independent of RANKL-RANK signaling³. The Bruton's tyrosine kinase (BTK) signaling pathway plays a pivotal role in inflammatory response and bone resorption⁴. Thus, targeting BTK may be efficacious against not only inflammation but also bone erosion by regulating activation of effector cells such as B cells, macrophages and osteoclasts in RA. We have already shown the inhibitory effects of TAS5315 on RANKL-dependent osteoclast activation. However, it remained uncertain whether TAS5315 inhibits osteoclast activation induced by inflammatory cytokines.

Objectives: In this study, we evaluated the effects of TAS5315 on osteoclast activation induced by inflammatory cytokines (*in vitro*) and the infiltration of tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts in the joint of a mouse CIA model.

Methods: *In vitro* biochemical assay was performed using available kinase assay panels. The BioMAP® Diversity PLUS panels were used to determine the profile of TAS5315 in primary human cell systems. The effects of TAS5315 on osteoclasts were assessed by examining osteoclast-mediated bone resorption. TAS5315 was orally administrated once a day in an established mouse CIA model. TRAP-positive osteoclasts were counted manually. Bone mineral density (BMD) and bone erosion were assessed using micro-CT analysis. The mechanical properties of the tibia were evaluated by a compression test of proximal metaphysis using a material-testing machine.

Results: TAS5315 selectively inhibited the enzyme activity of BTK and had less off target inhibition against other kinases. In BioMAP® systems, TAS5315 decreased the production of IgG and the expression of cytokines (TNF- α , IL-6, IL-17A). TAS5315 also inhibited osteoclast-mediated bone resorption induced by inflammatory cytokines. On the other hand, anti-RANKL antibody did not inhibit bone resorption induced by inflammatory cytokines. Furthermore, in the mouse CIA model, TAS5315 significantly ameliorated paw swelling and pathological changes. TAS5315 significantly decreased the infiltration of TRAP-positive osteoclasts in the joint and also showed improvement of BMD and bone erosion by time-dependent micro-CT analysis. In vehicle-treated mice, the mechanical strength of tibia was decreased compared with normal mice. TAS5315-treated mice recovered the decreased parameters of the mechanical strength compared with vehicle-treated mice.

These data suggests that TAS5315 improves bone erosion in murine model for RA through direct inhibition of osteoclast activation induced by inflammatory cytokines as well as RANKL.

Conclusion: Our study demonstrates that TAS5315 would be an attractive RA therapeutic drug that could improve bone destruction as well as inflammation.

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SAT0057

INCREASED MICRORNA-155 IS ASSOCIATED WITH A SPECIFIC DEFECT OF ANTI-INFLAMMATORY M2 MACROPHAGES POLARIZATION BOTH IN HUMAN RHEUMATOID ARTHRITIS AND IN COLLAGEN-INDUCED-ARTHRITIS MICE

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Background: Monocytes/macrophages are key players in the pathogenesis of Rheumatoid arthritis (RA). Monocytes can differentiate into pro-inflammatory macrophages (M1-like) or into anti-inflammatory macrophages (M2-like). We previously found a defect of monocytes polarization into M2-like macrophages, in RA patients and not in healthy donors (HD) or in patients with other inflammatory diseases (such as connective tissue diseases or spondyloarthritis), and a propensity for preferential maturation towards M1-like pro-inflammatory macrophages that could contribute to synovial inflammation.

MiR-155 expression has been shown elevated in RA synovial macrophages and in blood monocytes. MiR-155 inhibits mRNA of 2 important proteins involved in programming of M2 macrophages (SOCS-1 and C/EBP- β).

Objectives: We assessed if miR-155 could play a role in the defect of monocytes polarization into M2-like macrophages both in human RA and in a mouse model of collagen-induced-arthritis (CIA) mice.

Methods: Monocytes were isolated from PBMCs for HD and RA by negative selection. Purified monocytes were transfected using **AMAXA technologies** with miR-155 mimic (for HD) or antagomiR155 (for RA) or controls miRs. Then monocytes were incubated in the presence of human serum (SAB) (M2-like) for 6 days. Expressions of total macrophages markers (CD11b and CD71), M2-like macrophage markers (CD163, CD206 and Arg-1), M1-like macrophage marker (iNOS) were evaluated by flow cytometry, ELISA or immunofluorescence.

A. Monocytes from healthy donors

B. Monocytes from RA patients

C. Monocytes from CIA or control mice

DBA/1 mice were immunized by intradermal injection at day-0 and at day-21 with a grade chick type II collagen and complexe Freund's adjuvant. Monitoring of arthritis indices was done from day-25 to day-35. At day-35, monocytes were isolated from bone marrow by negative selection. To generate MDMs, purified monocytes were incubated 6 days in the presence of M-CSF+IL-4 (M2-like). Expressions of total macrophages markers (CD11b and F4/80), M2-like macrophage polarization markers (CD206, IL-10 and Arg-1) were evaluated by flow cytometry, ELISA or qPCR.

Results: Overexpression of miR-155 in HD monocytes significantly decreased the gMFI of the pan-macrophages markers, CD11b and CD71, but also the M2-like markers, CD206 and CD163 after 6 days of culture with human SAB. Likewise, we demonstrated a significant reduction of Arg-1 expression and increase in iNOS expression in M2-like macrophages after miR-155 transfection of HD monocytes (Fig. 1A).

Conversely, ex vivo silencing of miR-155 with antagomir in RA monocytes restored the defect of M2-like phenotype macrophages by increasing the percentage of pan-macrophages markers, CD11b and CD71 and M2-like macrophages markers CD206 and CD163 after a 6-day culture in human SAB. Moreover, miR-155 antagomir increased significantly the Arg-1 expression and reduced iNOS expression in M2-like macrophages (Fig. 1B).

The defect of monocytes capacity of differentiation into M2-like phenotype was also found in CIA mice as compared to controls, with a decreased expression of CD206, IL-10 secretion and Arginase-1 expression (Fig. 1C). MiR155 was also involved in this defect in mice: its expression level was the same in M1-like macrophages between controls and CIA mice, whereas it was increased in M2-like CIA macrophages compared to controls mice (Fig. 1C).

Conclusion: MiR155 plays a key role in the specific defect of anti-inflammatory M2 macrophages polarization found both in human rheumatoid arthritis and in CIA mice. MiR155 could represent a new therapeutic target in RA that can be tested in the mouse model of CIA.

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SAT0058

DEFICIENT DNA DAMAGE RESPONSE AND REPAIR IN ACTIVE RHEUMATOID ARTHRITIS AND THE EFFECT OF TREATMENT

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Background: Increased endogenous DNA damage poses a serious threat to cell health, since it may lead to mutagenesis, genomic instability and cellular apoptosis. DNA damage accumulation in immune cells occurs in patients with chronic inflammatory diseases, however; little is known on the types and prevalence of endogenous DNA damage in patients with Rheumatoid arthritis (RA).

Objectives: To study DNA damage response and repair network in patients with active RA and to test the hypothesis that anti-rheumatic treatment influences this network.

Methods: Peripheral blood mononuclear cells were isolated from 15 patients with active RA and 65 apparently healthy controls; 8 patients were re-examined after 12-week treatment. Endogenous DNA damage was determined using alkaline comet assay. Specific markers for single-strand DNA breaks (SSBs; RPA32) and double-strand DNA breaks (DSBs; γH2Ax, 53BP1) were measured using western-blot. Formation of DNA damage was assessed by oxidative stress and abasic DNA sites measurements. Chromatin organization and the two subpathways of the fundamental nucleotide excision repair mechanism, namely, transcription-coupled repair (TCR) and global genome repair (GGR), were assessed along the N-ras gene.

Results: A 3-fold increase of endogenous DNA damage levels (Olive Tail Moment reflecting both SSBs and DSBs) was evident in active RA [mean±SD; 12.8±7.5 versus 4.5±2.4, p<0.001], as well as induction of RPA32, γH2Ax and 53BP1, higher oxidative stress levels and increased abasic sites, compared to controls. While TCR capacity was preserved, GGR capacity was deficient in all active RA patients. Moreover, a more condensed chromatin structure was found in active RA compared to controls. Following treatment, chromatin structure loosened, GGR capacity was restored, oxidative stress and abasic sites decreased and levels of endogenous DNA damage reached control values in all patients.

Conclusion: Deregulated chromatin organization, deficient DNA repair capacity and augmented formation of DNA damage contribute to the accumulation of endogenous DNA damage in patients with active RA and are reversible after treatment. Additional studies to better understand the negative impact of DNA endogenous damage accumulation may create new therapeutic opportunities for patients with RA.

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SAT0059

APOLIPOPROTEIN B DERIVED PEPTIDES INHIBIT PRODUCTION OF PRO-INFLAMMATORY CYTOKINES IN PBMC OF RHEUMATOID ARTHRITIS (RA) PATIENTS AND IMPROVE ARTHRITIS IN ANIMAL MODEL OF RA

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Background: Alpha-enolase (ENO1) is a multifunctional glycolytic enzyme. The cell-surface expression of ENO1 is increased in monocytes isolated from peripheral blood mononuclear cells (PBMCs) of RA patients. In previous experiments, we reported apolipoprotein B (apoB) to be as a novel ligand of cell surface expressed ENO1 and to enhance chronic inflammation in RA (1).

Objectives: This study was performed to evaluate agonistic or antagonistic function of peptide sequences within apoB, which modulated severity of arthritis in K/BxN serum transfer arthritis mouse model.

Methods: Peptides binding to ENO1 were evaluated by peptide microarray. Peptides were designed to have twelve amino acids lengths with ten amino acids overlap. Levels of pro-inflammatory cytokines produced by PBMCs were measured after treatment with peptides in RA patients and healthy controls (HCs). To evaluate the antagonistic function of peptides, RA PBMCs pre-treated with peptides were stimulated with apoB and pro-inflammatory cytokines were measured in culture supernatant. Peptides were injected in K/BxN serum transfer mice and we measured the thickness of ankles and arthritis score to determine whether peptides could reduce arthritis severity.

Results: Among eighty five peptides from apoB, five peptides exhibited strong binding to ENO1. Two peptides increased production of pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α, but three peptides did not change levels of pro-inflammatory cytokines in RA and HC PBMCs. However the three peptides reduced production of pro-inflammatory cytokines in RA PBMCs stimulated with apoB. These three peptides were named IP (inhibitory peptide)1, IP2 and IP3. IPs were injected after induction of the arthritis in K/BxN serum transfer mice in vivo. Ankle thickness and arthritis scores were substantially reduced in IPs treated group than control group, and synovial inflammation, bone erosion and cartilage damage of arthritis were less severe in the IPs-treated groups. Especially, IP2 showed the most significant effect.

Conclusion: The apoB derived peptides interacting with ENO1 could have novel therapeutic effect in RA.

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